VOLUME XXXII NUMBER 1

BOTANICAL GAZETTE

JULY, 1901

ON THE ORIGIN AND NATURE OF THE MIDDLE LAMELLA.

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THERE has long been recognized in the cell walls of plant tissues, and particularly in the thickened walls of bast and xylem elements in woody plants, a central layer or plate, sharply distinguishable by its optical and staining properties from other regions of the wall. To account for the origin of this layer, apparently homogeneous and equally closely related to each of the cells between which it occurs, various theories have been advanced, and the names "primary cell wall," "intercellular substance," and "middle lamella" indicate the diversity of opinion that has prevailed as to its real nature.

The theories of the origin of this "middle lamella," adopting as most convenient its commonest designation, may (1), generally speaking, be grouped under three heads: (1) those which hold it to have been originally a common matrix in which the cells were imbedded; (2) the theory that the middle lamella is the original cell wall, laid down in common by the two daughter cells in the process of cell division, and remaining distinct from the secondary thickening layers deposited upon its either face; and (3) the view that it is a substance excreted by the daughter cells into a space left between them after their formation, or into a space formed by their rounding up and drawing apart.

The first theory, that the middle lamella is an original common matrix of the cells, was shown to be groundless as soon as

any satisfactory investigation of the process of cell division could be made.

The notion of the middle lamella as the primary cell wall until very recently was the most prevalent, and is the one still laid down in most text-books of botany. Vines (**15**) thus states this view:

> In the development of a tissue, whether by free cell formation or by cell division, septa are formed, that is, walls which are common to contiguous cells; these are very thin at first, and appear under the highest magnifying power as a simple plate. As the walls increase in thickness and acquire a more or less distinctly stratified structure, as seen in transverse section, the network of primary septa stands out from the thickening layers proper to each cell. The primary septum between any two cells is now distinguished as the *middle lamella* (sometimes also termed *intercellular substance*); it attains a considerable bulk at points where several septa meet at an angle.

This theory was early stated by Strasburger, and until lately he has adhered to it in unmodified form. In the first edition of the *Botanisches Practicum* (**10**), on p. 78, he describes a method of demonstrating the presence of this median layer in the endosperm of the seed of *Ornithogalum umbellatum* by treatment with sulfuric acid, the delicate network of the middle lamella being the last part of the cell walls to dissolve. This network, he says, corresponds to the original walls present before the process of thickening began. In another passage, on page 82, speaking of the tracheids of *Pinus silvestris*, he uses the term "primary wall," which, however, is not the same as the first wall laid down in cell division—the middle lamella—but includes the latter as a very delicate layer (*Theilplatte*). The middle lamella differs from the rest of the primary wall in being "cutinized." To the original partition laid down in the process of cell division, Strasburger (**9**) had in 1875 given the name "cell plate."

Treub (**14**), in 1878, studying the living cells of the proembryo of *Orchis latifolia* and the ovules of *Epipactis palustris*, found, after its formation, a splitting of the cell plate, and the deposition of a cell wall between the two layers so formed. This is, I believe, the first suggestion that the median layer of the cell wall is not the plate first laid down in the process of cell

division, but is a substance secreted into a space between the daughter cells.

The history of the cell plate theory has been carefully summarized by Timberlake (**13**), and reference may be made to his paper for the literature of the subject. The results of recent investigations go to show the essential correctness of Treub's view. In 1898, Strasburger (**12**) announced that the cell plate splits to form a plasma membrane for each daughter cell. During the splitting process, the rod-shaped elements visible in the cell plate, which represent the material furnished to the plate by the spindle fibers, are pulled out so as to become extremely thin in their middle portion. A middle layer then appears between the halves of the original layer. Whether or not the thread-like portions of the rods persist, either as part of this new layer or as protoplasmic connections between the plasma membranes of the daughter cells, he did not determine. So the question was left open whether the substance of the wall arises entirely by secretion into the space formed by the splitting of the cell plate, or whether a portion of the original cell plate takes part in its formation; but Strasburger has wholly abandoned his old notion of the identity of the middle lamella with the original cell plate.

Timberlake (**13**) finds in the cell divisions of the root tip of *Allium Cepa*, before the appearance of the cell plate elements, an accumulation of an orange-staining substance in the equatorial zone of the spindle. From the staining reactions of this substance, he concludes that it is some form of carbohydrate, probably destined for the building of the wall between the daughter cells. But, while in general the material of this zone stains like the cell wall, it is not stained, as is the wall, by either ruthenium red or iron hæmatoxylin. The cell plate is formed in the midst of this zone by the fusing together of equatorial thickenings of the spindle fibers. The carbohydrate material disappears with the formation of the cell plate, but very soon after the splitting of the plate the young cell wall (middle lamella) appears in the cleft. The cell plate begins to split in its oldest part, that is, in the central portion of the spindle, so

that a cell wall may be visible here before the cell plate by peripheral growth has reached the mother cell wall.

Mangin (**3, 4, 5, 6, 7, 8**), in a series of contributions which appeared from 1888 to 1893, investigated particularly the chemical nature of the substance in question. As this side of the subject and Mangin's very interesting conclusions regarding it have been little discussed, a somewhat full résumé of his results may here be given.

Dippel, quoted by Mangin (**3**), had already announced that the new partition deposited by the protoplasm has none of the properties of cellulose. Frémy found in the tissues of fruits and roots a substance called by him pectose, which he was unable to separate from cellulose, and from which are produced the pectic compounds found in the walls of fruits. Maudet found in the pith of certain trees pectose and calcium pectate, forming a cement which holds the cells together. Mangin (**3**) found plant cell walls to be generally formed by the association of two substances, cellulose and one which he provisionally called pectose. In a large variety of adult tissues he found pectose, in a pure state, forming the middle lamella or intercellular substance, and associated with cellulose in the other layers of the cell wall. Dippel's conclusion, that the middle lamella is free from cellulose, was thus confirmed. Some tissues were found to be composed wholly of pectose. It plays the principal rôle in what had been called cellulose fermentation, and Mangin considered chemical modifications of cell walls, as lignification and cutinization, to be due to transformations of pectose, of which cellulose is incapable. At this time (1888) he repeatedly speaks of the pectose layer as the first membrane formed in cell division, the "fundamental layer of the cellular membrane." This seems to indicate an acceptance of the view then held by Strasburger as to the identity of the middle lamella with the cell plate.

But in 1890, Mangin (**5**) suggested the appropriateness of restoring to this layer the name "intercellular substance," as expressing better than that of "middle lamella" its origin and

mode of formation. He found it to consist, not of pectose proper, but of a cement of insoluble pectates. To demonstrate the presence of pectic acid, tissues are macerated in a mixture of alcohol and hydrochloric acid, and then treated with a weakly alkaline solution. The tissues dissociate into their constituent cells and fibers, the intercellular substance passing into solution. The solution is shown by analysis to contain pectic acid, which appropriate staining now shows to be absent from the cell walls. In this process, the insoluble pectates are first changed into pectic acid, which, in the alkaline solution, forms a soluble alkaline salt. If sections first treated with acid alcohol are stained with a pectic stain, as phenosafranin or methylene blue, the pectic acid present stains more deeply than the pectic compounds associated with cellulose in the wall layers lying between the intercellular substance and the cell cavity. This indicates that it is not pectic acid and its derivatives, but some of the neutral pectic substances, as pectin or pectose, which are in combination with cellulose in the later deposited layers.

The intercellular substance thus deeply stained forms a thin layer on the whole surface of contact of adult cells; where the cells draw apart, it produces a thick cushion; when the cells separate so as to form intercellular spaces, these spaces are bounded by a pectic layer. This frame of pectic acid is sometimes thickened irregularly, so as to form knobs, points, and sculptures of various forms ornamenting the frame itself or projecting into the intercellular spaces. Sometimes the spaces are partially or completely filled with a jelly-like mass, a soluble transformation product of pectic acid. In the meristem the intercellular layer is not disclosed by staining, but the chemical reactions of cellulose and pectic compounds are given by the cell membranes. That a thin layer of intercellular substance is present is shown by the dissociation of the cells as in older tissues after treatment with acid alcohol and an alkaline solution. Mangin explains the variations in thickness and form of the intercellular cement by its partial transformation in the course of the development of the tissues into soluble pectates; this

makes possible the splitting of the membrane, the formation of intercellular spaces, and an exudation of material to form the knobs, points, and other structures already referred to.

A further proof of the presence of pectic compounds in the wall is furnished by treating tissues with a cellulose solvent; the general outlines of the cells remain, the middle lamella, and often a large part, or in some cases all, of the other wall layers preserving their form. The framework thus left takes up pectic acid stains and is indifferent to cellulose stains. The best distinctive stain for pectic substances is ruthenium red, the ammoniacal sesquichlorid of ruthenium. Our knowledge of the value of this subtance is due to Mangin (**8**), who first described its remarkable properties as a staining reagent for vegetable tissues.

Mangin's attention, it is seen, has been directed to the chemical composition and transformations of the "intercellular substance," and he has not connected his valuable results obtained in that field with a study of its origin. It was in part to supplement his work in this important regard that the present investigation was undertaken.

Strasburger (**12**) and Dippel (**2**) accept Mangin's conclusions as to the widespread presence and the importance of pectic substances in plant tissues, especially in the middle lamella and the thin layer immediately surrounding the intercellular spaces. Dippel's account of the history of the middle lamella in the wood and bast of higher plants is briefly as follows (pp. 570 ff.).

The radial walls of dividing cambium cells are separated from one another by a lax, weakly refractive *Zwischenmasse* or *Zwischensubstanz*. This substance decreases in amount as we pass outward from the cambium layer into the differentiated wood and bast tissues, until it finally disappears, excepting at the angles formed by the junction of three or four cells. Remains of the cambium mother cell walls may occasionally be recognized in the *Zwischensubstanz*, which Dippel believes to be at least partially produced by their disorganization. Chemically, the *Zwischensubstanz* may consist of a union of pectose and callose, which is soluble under the conditions of cell wall development,

and so, for the most part, is absorbed into the cambial daughter walls. The cambial cell walls (p. 575), that is the cell walls of the cambium mother[1] and daughter cells, are composed of pectic acid, which, at least after the transformation of these walls into an "intercellular substance," exists largely in the form of calcium pectate. As the cambial daughter cells are transformed into bast and wood tissue, the primary cell walls, consisting of pectose and cellulose, are deposited next to the now apparently simple cambial walls. These latter now undergo a transformation, forming an "intercellular substance," or *Kittsubstanz*, which, through a loss of water and the extension in length of the radial walls, becomes so thin as to be in most cases invisible except after special treatment. It is, besides, fused into apparently intimate connection with the primary cell walls. This combination of the intercellular substance and the primary cell walls forms the middle lamella of mature tissues, against which in the process of development secondary thickenings are deposited. The continued existence of the "intercellular substance" as a middle plate (*Theilplatte*) of the middle lamella Dippel demonstrates by the action of the wall layers upon polarized light, as well as by treatment with various macerating and staining reagents, notably ruthenium red.

Dippel's peculiar view, developed in connection with his studies on the algae, that cell division is accompanied by the formation of a new cell wall entirely enclosing each daughter cell, has doubtless influenced his account of the development of the middle lamella in the higher plants. For if this were the case in the predominantly radial growth of cambium cells, the result would be to leave, between the radial walls of the newly-formed daughter cells, the old mother cell walls, which, if they became disorganized, would form a *Zwischensubstanz* such as Dippel describes. The tenability of this view of the origin of the *Zwischensubstanz* will be discussed hereafter.

[1] Here there seems to be a contradiction, for Dippel has already spoken (p. 575) of the cambium mother cell walls as being disorganized to form the *Zwischensubstanz* of the daughter cell walls.

MATERIAL AND METHODS.

Woody tissues of the following species were studied: *Pteris aquilina* L., *Pinus silvestris* L., *Nerium Oleander* L., *Rosa* sp., *Tilia Americana* L., and *Ilex opaca* Ait. Of these, Mangin had investigated tissues of Pinus and Ilex.

Free-hand sections were cut, usually both transverse and longitudinal. In many cases, before staining, they were treated for twenty-four hours, as suggested by Mangin (**5**), with a mixture of one part of concentrated hydrochloric acid and four or five of absolute alcohol, then washed in distilled water. The stains characteristic of pectic acid, especially ruthenium red, are taken up, as a rule, more freely after this treatment, and almost invariably the coloration is more clear-cut and distinctive.

For staining, Grübler's preparations were used. Unless otherwise stated, they were in neutral saturated solution. When an alkaline solution was used, it was prepared by adding to the neutral solution about ½ per cent. of concentrated ammonia solution. Acidulated solutions were prepared by adding a like proportion of acetic acid. In some cases where saturated solutions, *e. g.*, of methylene blue and methyl violet B, were too strong for good results, they were diluted to such a strength that a few seconds' exposure gave a differential stain. Aniline-water-safranin was prepared as directed by Zimmermann (**16**). An aqueous solution of ruthenium red of a strength of about one five-thousandth was used. An increase in strength above this did not seem to make any noticeable difference in the depth of the stain or in the time of exposure required. This substance is reduced by the action of light in the presence of water, hence the solution must be kept in the dark. It was tried in neutral, alkaline, and acidulated solutions, the alkaline and acidulated solutions being prepared as described for the other coloring matters, substituting hydrochloric for acetic acid. In all cases, the alkaline solution was found to give the deepest and most distinctive colorings, and it is this preparation of ruthenium red that is referred to hereafter. But a few minutes' exposure to the stain is required.

In general, sections after staining were mounted in water and studied immediately. Ruthenium red preparations can be dehydrated by absolute alcohol, passed through clove oil, and mounted in Canada balsam without affecting the stain. But the color gradually fades out from sections so treated, some tissues, notably the xylem and bast, losing their color almost entirely within a few weeks, while others, as the parenchyma and pith, retain it much longer. Sections stained with ruthenium red and mounted in glycerin jelly lose their color almost entirely in the course of a few hours.

Mangin (**4, 6, 7, 8**) has classified the coloring matters used in staining plant tissues, and has described their reactions upon the various substances found in cell walls. Of the great number of stains named by him, the following were employed in this investigation:

Orseille, which colors cellulose in neutral or slightly acidulated solution, but does not affect callose.

Aniline-water-safranin (phenosafranin), methylene blue, Bismarck brown (Vesuvian brown), fuchsin, and methyl violet B (violet de Paris), which do not color callose or cellulose, but do color pectic acid in neutral or acidulated solution. They also color equally well nitrogenous substances (lignin, cutin, etc.), but these may be distinguished by their retention of the color after treatment with alcohol, glycerin, or acids, which treatment decolorizes pectic acid. According to Strasburger (**11**), safranin colors the protoplasmic contents of the cell, the lignified wall, cork, and cutinized membranes cherry-red, the pectic substances orange-yellow. Methylene blue colors the protoplasmic contents and lignified walls blue, the pectic substances violet-blue.

Acid brown, nigrosin, and ponceau, which do not color pectic compounds, but strongly color nitrogenous substances. By mixing one of this series with one of the preceding, Mangin obtained distinctive double stains.

Ruthenium red, which Mangin found the most satisfactory distinctive stain for pectic compounds. It differs from the other pectic stains, as safranin and methylene blue, by the fact that

pectic compounds stained by it are not decolorized by glycerin or alcohol. Ruthenium red, he finds, also stains gums and mucilages formed by the decomposition of pectic substances, but does not affect decomposition products of cellulose or callose. Lignified tissues, not stained by ruthenium red when fresh or preserved in alcohol, take it up after treatment with alkalies or Javelle water; but their affinity for ruthenium red is always less than for certain basic organic stains, such as methylene blue, so that by combining its action with that of one of the latter class fine double colorations may be obtained. Cutinized membranes, according to Mangin, are stained in many cases, but not the cuticle. Ruthenium red also stains in varying degrees the protoplasmic cell contents.

PTERIS AQUILINA.

The rhizome was studied in cross section. In unstained sections the middle lamella of the sclerenchyma walls is plainly distinguishable from the inner layers by its greater density and refractive power. It appears as a dark yellowish-brown layer constituting a considerable proportion of the total thickness of the wall, enlarged at the angles where three or four cells abut into a triangular or quadrangular form, often enclosing at such places a similarly shaped intercellular space.

In the walls of the stone cells, while the absolute thickness of the middle lamella is approximately the same as in the sclerenchyma, its thickness relatively to that of the whole wall is of course considerably less. Otherwise its appearance is much the same as in the sclerenchyma. The canals appear as transverse lines seeming to pass quite through the wall, including the middle lamella.

On treatment with methylene blue, the sclerenchyma wall is colored green, except the middle lamella, which, if stained at all, yet appears yellowish-brown in contrast with the other layers. The walls of the fundamental parenchyma, as well as the protoplasmic cell contents, are stained blue. The parenchyma walls are apparently continuous with, and of the same thickness

as, the middle lamellae of the sclerenchyma. The green appearance of the sclerenchyma walls is probably not a differential coloring, but is due to a combination of their original yellowish-brown with the blue stain.

In sections treated with ruthenium red, the sclerenchyma and stone cell walls do not take up the stain to any appreciable extent. The walls of the fundamental parenchyma are clearly brought out, and are again plainly continuous with the middle lamellae of the sclerenchyma and stone cells. The indifference of the middle lamella in these thickened walls to methylene blue and ruthenium red, although this layer is directly continuous with the thin, readily-staining parenchyma wall, is in marked contrast to its character in the tissues of the spermatophytes studied; it may be due to a change in the chemical composition of the middle lamella which occurred simultaneously with the deposition of secondary thickening layers. The fundamental parenchyma walls show no evidence of secondary thickening. They are stained by ruthenium red deeply and quite uniformly, save that at the angles the wall is thicker, the triangular or quadrangular area so formed is less deeply stained, and contains apparently a less dense substance than that forming the remainder of the wall. This less deeply stained area corresponds to the thickened corners and the intercellular spaces of the sclerenchyma. The protoplasmic contents of the parenchyma cells are stained unevenly, the nuclei very deeply.

In the vascular bundles, the parenchyma walls are deeply and uniformly stained by ruthenium red. These walls appear continuous with the stained middle lamellae of the large vessels and sieve tubes. In the walls of the vessels the middle lamella is relatively thin; the rest of the wall is uncolored. The middle lamella of the sieve tubes occupies a larger proportion of the wall thickness, the interior unstained layers being very thin. At corners formed by three or four cells the middle lamella appears more dense and somewhat, though not greatly, enlarged.

The staining reaction of the middle lamella in these elements is in contrast to its conduct in the sclerenchyma and stone cells,

and agrees with results generally noted in tissues of spermatophytes.

PINUS SILVESTRIS.

Sections of young twigs and branches cut in the fall were used; these, therefore, were past the season of rapid cambial division, and the cambium cells were in a condition of comparative rest. Sections of old and seasoned pine wood were also used for comparison with the young tissues.

In cross sections through the cambium and neighboring tissues of young wood, treated with acid alcohol as above described, the cambium walls are stained throughout their thickness by methylene blue a deep and apparently uniform blue. The result is similar in sections not previously treated with acid alcohol.

Similar cross sections, both in the fresh condition and after treatment with acid alcohol, were stained with ruthenium red. This stain is also taken up freely by the cambium walls. The radial walls are noticeably thicker than the tangential. In the middle of the radial walls there is often a less deeply staining layer, recognizable as Dippel's *Zwischensubstanz*. The rest of the walls are deeply stained, except that in many of the older cambium cells, whose walls are thicker than those of the youngest cells present, there is, next the cell interior, a light line indicating a later deposited, unstained, or less deeply stained wall layer. The corners where radial and tangential walls join are especially deeply stained. In many cases, a continuation of the radial cambium wall into the middle lamella of the xylem and bast can easily be traced.

A difference in depth of stain between radial and tangential walls similar to that given by methylene blue is found in the longitudinal sections treated with acid alcohol and exposed for a few minutes to the action of Bismarck brown.

In cross sections treated with acid alcohol and stained one hour with fuchsin, the cambium walls are uncolored; the protoplasmic contents of some of the cambium cells are stained red. The difference between the affinity of these walls for Bismarck

brown and fuchsin is worthy of note; both these stains belong to the class in which a basic coloring matter is united to an acid; according to Mangin, all the stains of this class stain pectic compounds and do not color cellulose or callose.

In unstained cross sections through the xylem, the middle lamella is to be distinguished from the rest of the wall by its different refractive power, at some levels of focusing appearing brighter, at other levels darker, than adjacent layers. The same is the case in cross sections through old pine wood, except that here the middle lamella appears, if anything, more dense than in the young wood.

In cross sections through young wood treated with acid alcohol and methylene blue, the whole xylem is colored green. The middle lamella is distinguishable only by its greater density, as also in sections not previously treated with acid alcohol.

Xylem walls in cross sections treated with acid alcohol stain more deeply with ruthenium red than in sections not so treated. The middle lamella stains much more deeply than the inner layers, the boundary between the lightly and deeply stained portions being sharply marked. The difference in depth of stain is much more apparent in acid alcohol sections than in the others. The tori of the pits in acid alcohol sections are stained deep red; this occasionally occurs also in sections not treated with acid alcohol. In some cases in acid alcohol sections, the torus appears plainly to be a continuation of the middle lamella. In some cases also the middle lamella appears continuous with the interior layer of the pit cavity, which stains deeply. The middle lamella is more plainly differentiated in xylem of the present year's growth. In some preparations the oldest cells of the present year's growth (the spring wood) have the whole wall quite deeply stained. These facts may indicate that it is in the younger xylem walls that pectic substances exist in purest form. Where splitting of the walls occurs in preparing the section, it is sometimes on the line of demarcation between the middle lamella and the less stained layers; very often, however, the split is approximately through the center of the middle

lamella. Intercellular spaces occur in the xylem where three or four cells abut; they appear as triangular or quadrangular breaks in the uniformly stained middle lamella.

Comparing the results obtained from methylene blue and ruthenium red, we find that by both the whole xylem wall is stained, the middle lamella being differentiated by the red and not by the blue. Since methylene blue stains nitrogenous substances equally well with pectic acid, and does not stain cellulose or callose, while ruthenium red stains pectic derivatives and not nitrogenous substances, it appears that the secondary layers of the xylem, as well as the thin layer of the cambium wall which does not take up ruthenium red, contain a mixture of nitrogenous and pectic compounds. The use of orseille, a cellulose stain, gave no satisfactory results, showing that celulose, if present at all in these walls, is of minor importance. The staining power of the tori of the pits indicates that they represent in the main the same layer as the middle lamella; this does not, however, preclude the possibility of their also possessing a thin, subsequently deposited layer. The deeply stained layer lining the pits is an example, of which others will be mentioned later, of the possible deposition of a pectic layer very late in the history of the growth of the cell wall.

The relations of the middle lamella, tori, intercellular spaces, and thickening layers appear about the same in older pine wood stained with ruthenium red as in the young tissues already described.

In cross sections previously treated with acid alcohol and exposed for one hour to fuchsin, which belongs to the same class as methylene blue, the xylem walls are stained throughout. Other tissues, except the contents of the cambium and collenchyma cells, are unstained.

Cross sections of older pine wood were left in ponceau solution for three or four days. The xylem is quite uniformly stained vermillion, the middle lamella generally appearing darker than the inner layers of the wall, but the distinction of coloration is not strongly marked, and the difference may be wholly due to the difference in density.

The xylem walls of young wood are not stained after a half-hour's treatment with acidulated nigrosin solution. This fact, by itself, would mean the absence of nitrogenous substances; but, as against that furnished by methylene blue, and by ponceau, which belongs to the same class as nigrosin, such negative evidence is of little value.

In longitudinal sections of young tissues treated with acid alcohol and stained with ruthenium red, the tori of the pits are stained deep red. The appearance of the tori and of the red-staining bars between the pits, which bars are referred by Dippel (2) to the remains of the *Zwischensubstanz*, is well shown by him in his figure 397. In many cases, probably in partly developed pits, the stain is only a deep spot at the center. In some cases, an unstained center is surrounded by a stained ring. The pits are not so noticeable where they connect with the cells of the medullary rays, but where the ring appearance just described is found in older wood, the tori of pits so situated are completely stained.

Both the walls and the cell contents, especially the nuclei, of the medullary rays are deeply stained by methylene blue, both in cross sections previously treated with acid alcohol and in those not so treated. This is true of both young and old wood. Similar results are effected by ruthenium red. In cross sections of old wood stained with ruthenium red and then exposed for a few seconds to methylene blue, the red stain of the xylem is replaced by the blue, while, if the action of the blue has not been too long continued, the medullary rays remain red. The greater affinity of the xylem walls for the blue than for the red is due probably to their containing nitrogenous and pectic compounds, both of which take up methylene blue, while the former, unless specially treated, have no affinity for ruthenium red.

In cross sections treated for one half hour with acidulated nigrosin solution, the medullary rays and their cell-contents take up the stain freely.

In cross sections of older pine wood treated for three or four days with ponceau, the medullary rays are unstained.

The readiness with which the walls of the medullary rays take up methylene blue and ruthenium red, and also nigrosin, indicates the presence in them of both pectic and nitrogenous substances. Their preference for ruthenium red indicates a greater proportion of pectic constituents than in the xylem walls. But against this view is the negative evidence of the ponceau, though this, perhaps, is not of much weight.

The cells about the resin pits and their contents, if any, are always deeply stained by either methylene blue or ruthenium red. This indicates, according to Mangin, that the resin and other substances formed in the breaking down of these cells are wholly or chiefly decomposition products of pectic acid and its derivatives.

The staining of the bast is in general similar to that of the xylem. In both acid alcohol and fresh sections, the whole wall takes up more or less freely either methylene blue or ruthenium red, the middle lamella being much more deeply stained than the inner layers. The middle lamella, of course, is thicker relatively to the total thickness of the wall, but is of about the same absolute thickness as in the xylem.

The middle lamella of the collenchyma walls is generally stained more deeply than the other wall layers by methylene blue or ruthenium red, especially in sections treated with acid alcohol. Here, except at the corners, the unstained portion of the wall is very thin, and often the whole wall appears quite uniformly stained. The stain appears more diffuse than in the xylem, and the distinction between the more and less deeply stained layers is not so sharp. The middle lamella is enlarged at the angles of the cells, and sometimes encloses a triangular or quadrangular intercellular space.

The appearance of the collenchyma is very much the same in longitudinal as in cross sections. The collenchyma walls become red when treated for thirty minutes with acidulated orseille solution, indicating the presence of some cellulose, but no distinctive stain for different portions of the wall is observable.

The walls, except the outer ones, of the epidermal cells in

either acid alcohol or fresh sections are deeply stained by ruthenium red throughout their whole thickness, but the cuticle remains unstained. This accords with Mangin's results on cutinized membranes.

NERIUM OLEANDER.

Cross sections were cut through growing stems of various ages. The whole of the cambium wall is deeply and quite uniformly stained by ruthenium red either after or without previous treatment with acid alcohol. Similar results are attained by a few seconds' exposure to dilute methylene blue solution. The cambium walls are not stained by an exposure of three or four minutes to acid brown. The middle lamella of the xylem is deeply stained by methylene blue or ruthenium red, the other layers of the wall being colored very slightly if at all. The xylem walls are colored blue by methyl violet B. They are stained quite uniformly brown in a few minutes by acid brown. They are stained uniformly red in forty-five minutes by aniline-water-safranin. After thirty minutes' exposure to this safranin solution the xylem walls are colored bright red, the middle lamellae between the large tubes being decidedly darker than the thickening layers. After forty minutes' exposure to ponceau, the thickening layers of the xylem are stained somewhat, though not evenly, the middle lamella remaining uncolored.

Sections were exposed for twenty-four hours to neutral, acidulated, and alkaline solutions of orseille. The neutral and alkaline solutions produced the same coloration of the various tissues as the acidulated, but to a less degree. Therefore only the action of the acidulated solution will be described. The coloration of the xylem and medullary rays is a purplish-red, of all the other tissues affected, brown. The walls of the xylem have a general purplish tinge, but no distinctive staining is apparent in the different layers. The contents of the medullary ray cells are deeply colored, and the walls are stained similarly to those of the xylem. An exposure of two and one half hours to ponceau produces a reddish tinge in the xylem walls, and no further stain.

The contents of the medullary ray cells are stained by acid brown. Both the walls and the cell contents are deeply stained by methylene blue as well as by ruthenium red.

The results as to the tissues just described agree closely with those obtained for Pinus. The cambium walls and the middle lamellae of the xylem and medullary ray walls are of pectic nature; the remaining layers of the xylem and medullary ray walls are composed of a mixture of pectic and nitrogenous substances, the pectic constituents being perhaps predominant in the medullary rays; and there is evidence of the presence of a small proportion of cellulose in these thickening layers.

The bast fibers, when treated with ruthenium red, show a peculiar and most beautiful differential stain. It is most clearly brought out after treatment with acid alcohol, though it appears in sections not so treated. The middle lamella, which is relatively very thin, is deeply stained. It is sharply distinguished from the layer next within it on either side, which is colorless or very slightly stained. Next, passing toward the interior of the cell, comes a layer with a little color, then one a little more deeply stained, and so on, the depth of stain increasing until the last thickening layer, reaching almost to the center of the cell, is colored to about the same degree as the middle lamella. It should be noted that the stain shown in these walls is rather a purplish-red than the bright red commonly found in ruthenium red preparations. This behavior with ruthenium red is markedly different from that shown by Dippel (**2**) in his figure 139, in which all the wall of the bast fibers except the middle lamella is shown as unstained. On page 219, he mentions, as an instance of pectose-free cell walls, the bast fibers of *Nerium Oleander*. This is plainly an error.

The bast fibers are not stained at all by exposure for a few seconds to methylene blue. By methyl violet B (three or four minutes), the middle lamella of the bast and the other wall layers except those next the middle lamella are stained violet.

Acid brown has an effect exactly opposite to that of ruthenium red; that is, the middle lamella is not stained; the next layer is

quite deeply colored, the next is lighter, and the colors grow less and less intense until the innermost layers show no coloration at all. After a ten minutes' exposure to acid brown, sections were dehydrated, passed through clove oil, and mounted in Canada balsam, without affecting the staining, except that the color of the bast fibers was somewhat faded. The contents of the bast fibers are deeply colored by acid brown.

The action of orseille (acidulated solution, twenty-four hours exposure) is exactly like that of acid brown. The effect of aniline-water-safranin, on the other hand, is similar to that of ruthenium red.

Exposure to ponceau for various periods up to two and one half hours does not stain the bast.

By treatment for a few minutes with methyl violet B, the middle lamella of the bast and all the other layers except that next the middle lamella are stained violet.

It appears that in the bast, as generally elsewhere, the middle lamella is of pectic nature; that the first of the secondary layers is nearly or quite free from pectic substances, and is composed of a mixture of cellulose and nitrogenous substances, and that the subsequent layers gradually increase in pectic content at the expense of the cellulose and nitrogenous constituents, until the last layer is perhaps again purely pectic. But, from the purplish-red color given by ruthenium red and the indifference shown toward methylene blue, it may be inferred that the pectic compounds found in these walls are not exactly the same as those common to the middle lamellae of other tissues. These inner layers, like the pectic lining of the canals in the xylem of Pinus, show that the ability of the cell to secrete pectic acid is not limited to a single period of its development.

Acid alcohol sections treated for a few seconds with methylene blue show a deep blue color in the middle lamella of the collenchyma walls, much deeper than in the remaining portions of the wall, which, however, are also somewhat stained. The boundary between the middle lamella and the next adjoining layers is not so sharply defined as in the xylem and bast. At

the corners of the cells there is an angular, less deeply stained area enclosed by the middle lamella, just as in the fundamental parenchyma of Pteris.

The effect of ruthenium red on the collenchyma is similar to that of methylene blue. The collenchyma walls are not stained by acid brown (three or four minutes), but the cell contents are deeply colored. The contents are also stained brown by acidulated orseille solution, and the walls show a brownish tinge, but no distinctive stain for the different layers. The cell contents are stained by ponceau (forty minutes), but not the walls.

Acid alcohol sections treated for thirty minutes with aniline-water-safranin show a deeper stain in the middle lamella of the collenchyma than in the other wall layers.

The walls of the cork cells are not colored by methylene blue nor by acidulated orseille solution. Orseille also does not stain the cuticle.

In acid alcohol sections the middle lamella of the cork cells is stained more deeply by aniline-water-safranin (thirty minutes) than is the rest of the wall.

The pith cell walls take up ruthenium red freely, the middle lamella, which occupies the greater portion of the thickness of the wall, being deeply stained, especially at the corners. Sometimes the whole wall appears quite uniformly stained. The intercellular spaces are sometimes angular, but very often rounded, elliptical, or circular.

The contents of pith cells are stained by acid brown, the walls unstained. The action of orseille is similar. The failure of these two stains to affect the pith walls indicates that those walls are composed chiefly of pectic compounds, probably in purest form in the middle lamella.

ROSA SP.

In general, the results were the same as for Nerium. Cross sections were treated with acid alcohol and stained with ruthenium red. The cambium walls stain quite uniformly

throughout. The xylem shows a distinctive stain for the middle lamella, which is enlarged and especially deeply stained at the angles. The medullary ray walls and cell contents stain deeply.

The middle lamella and successive thickening layers of the bast fibers stain exactly as described for Nerium, including the purplish tinge. The stain of the middle lamella is deepest at the angles. The lining layer of the canals running from fiber to fiber is deeply stained where it traverses unstained or less deeply stained layers. This recalls the deeply stained layer of the pits of pine tracheids, and, with the deep stain of the last deposited wall layer in these tissues of the rose, shows these cells to possess the power very late in their history of depositing pectic wall material.

The pith cells show a very distinctive stain for the middle lamella.

In the collenchyma, the walls are stained throughout, the middle lamella much more deeply than the other layers, but the boundary between more and less deeply stained portions is not so clearly defined as in the xylem. The epidermal walls are stained about the same as the collenchyma. The cuticle is unstained.

Cross sections of a slightly older stem than that just described were stained with ruthenium red, then exposed for a few seconds to methylene blue. The xylem walls are stained purple by the combination, the middle lamella most deeply. The bast fibers take up both stains, the relative depth of stain of the various layers being about the same as with the red alone. The epidermal walls take up the blue more freely than the red. The collenchyma cells remain red.

Cross sections of a very young growing shoot were treated with acid alcohol and stained with ruthenium red. The cambium walls stained deeply throughout. Very thin tangential red lines are visible, representing the earliest deposit of wall material. Such very thin tangential walls are what one would expect to find in material gathered in the season of active cambial growth.

Young xylem elements have the walls entirely stained,

showing a long-continued period of pectic deposition. In older fibers a middle lamella is deeply stained, the remaining layers very slightly or not at all.

The bast fibers are stained throughout; no thickening layers are yet distinguishable. The middle lamella is already differentiated in the collenchyma, the other layers remaining uncolored. The middle lamella is especially deeply stained in the corners, and angular intercellular spaces are in some cases inclosed by it.

Similar sections not treated with acid alcohol yield similar results.

TILIA AMERICANA.

Cross sections through rather young growing shoots were treated with acid alcohol and ruthenium red.

The cambium walls stain deeply, in the youngest cells throughout their entire thickness. The radial walls are more deeply stained than the tangential, the corners especially deeply. Passing from the youngest cambium cells toward the bast or toward the xylem, the beginnings of the secondary thickening may be seen in a very thin unstained or only slightly stained layer of the wall within the stained portion. This is similar to the behavior of the cambium walls of pine, except that in Tilia, as generally, there is no evidence of a *Zwischensubstanz*, which, so far as I have observed, is found only in the pine cambium walls. The continuation of the deeply stained layer can be traced in the middle lamella of the much thickened xylem and bast elements.

The middle lamella of the xylem stains deeply, especially at the angles, where it is enlarged. The rest of the wall is slightly tinged with red.

The contents of the medullary ray cells are stained. Their walls are more uniformly stained than those of the xylem elements, but the middle lamella is still plainly distinguishable.

The bast has a deeply stained middle lamella, the other layers of the very much thickened wall being unstained or only slightly stained. In this respect, as in most others, the staining reactions of Tilia resemble those of pine.

The walls of the parenchyma cells between the bast bundles show a stain throughout, the middle lamella appearing thin and deeply stained, and somewhat enlarged at the angles.

In the collenchyma the whole wall and the cell contents are stained. Here, too, the middle lamella is thin and deeply stained, often including at the angles a less deeply stained area.

The walls of the cork cells stain quite uniformly throughout, but not very deeply. The color is purplish, different from the bright red typical of the middle lamella in other tissues. No middle lamella is here distinguishable. If anything, the innermost wall layer, next the lumen, is most deeply stained. Here, as in some of the tissues of Pteris, there is evidence that the middle lamella has undergone a chemical change, some or all of its pectic content having been replaced by other substances. The cuticle is unstained.

ILEX OPACA.

The tissues of the holly resemble those of the pine in their reactions to ruthenium red, the only stain I have used upon them.

In cross sections, either with or without previous treatment with acid-alcohol, the cambium walls are stained throughout by ruthenium red, and their connection may be traced with the middle lamellae of the xylem and bast.

The middle lamella of the xylem is thickened at the corners and stains deeply; the rest of the wall stains but slightly.

The middle lamella of the pith, which takes a deeper stain than the rest of the wall, is thickest at the angles, where it frequently encloses intercellular spaces. These spaces are sometimes triangular or quadrangular, but usually with more or less rounded angles, and often of an elliptical or circular form.

The middle lamella of the bast stains deeply, the rest of the wall slightly. The same is true in the collenchyma walls, where frequent angular intercellular spaces occur. The walls of the cork cells show no stain.

DISCUSSION.

In almost every tissue studied in which there was evidence of the deposition of wall layers at different periods, the staining reactions of the middle lamella showed it to differ in chemical composition from the adjoining layers; my results confirm Mangin's in every respect upon this point. Its distinctive character was brought out most clearly by ruthenium red. The reasons for concluding that, in general, this peculiarly staining layer is composed of pectic acid and its derivatives, have already been given at length. It does not follow, however, that its chemical composition is unchangeable. On the contrary, it is clear that, during cell growth and development, changes occur in the chemical constitution as well as in the form and mass of the middle lamella.

In pointing out the existence of a *Zwischensubstanz* between the radial walls of the cambial cells, and in distinguishing this layer from the "intercellular substance," either as characterized by Mangin or by himself, Dippel has contributed essentially to our clear conception of the conditions in the cambium of the pine; but his notion that the *Zwischensubstanz* is derived from the walls of the cambium mother cells has little evidence in its favor. I have never seen in this mass the fragments of these walls of which he speaks (**2**, p. 575). Besides, were Dippel's view correct, the tangential walls would be expected to contain at least as much of this substance as the radial walls; but, in fact, nothing of the sort is to be seen between the tangential walls, which, however, as shown in Dippel's own figures, are no thicker than are the radial walls without the *Zwischensubstanz*. On this point of the origin of the *Zwischensubstanz* Dippel is not clear, for he distinctly says (p. 575) that it is derived from the degenerated cambium mother cell walls, and again, on the same page, he speaks of the latter as going to make up the intercellular substance. It seems to me more probable that this *Zwischensubstanz* represents pectic acid which has exuded through the cambium cell walls into an intercellular cleft formed by the splitting of the radial walls, and that it is analogous to the lax substance

sometimes found at the angles of cells before the appearance of empty intercellular spaces. Its ultimate fate is doubtless to be absorbed into the adjacent cell walls, so that it does not appear as a visible layer in the middle lamella of mature tissues except in the case of the areas represented by the red-staining bars of the pine tracheids (see Dippel's figure 397), and except also, perhaps, on the surface of intercellular spaces. The intercellular spaces of the pine wood, then, are to be interpreted as remnants of the radial clefts between the cambium cells, rather than as newly formed in the adult tissue. But this does not preclude the probability of changes in the form and extent of the spaces due to the rounding up of the adjacent cells. It would be interesting to know whether this *Zwischensubstanz* is of wide occurrence. I have not observed it elsewhere than in the pine, and Mangin does not specifically note its existence.

A fact which is important as showing the plastic nature of the middle lamella at an early period of its history is its variation in thickness in different portions of older walls. This is shown commonly by an enlargement at the angles and a decrease in proportional thickness at the sides of the cells. Such variation is in marked contrast in the spermatophytes studied to the practical uniformity in thickness of the completely stained cambium walls. These are continuous with the middle lamella of the differentiated tissues, and, were it not for this change in form, would appear identical with the middle lamella. In some of the tissues examined, the substance of the middle lamella appears more dense at the enlarged angles, and seems to take up the characteristic stains more freely at those places. This appearance, however, may be accounted for by the optical effects produced by the greater thickness at the angles. But in other cases, as in the fundamental parenchyma of Pteris and the collenchyma of Nerium, these enlarged angles enclose a less deeply staining substance, or are becoming empty. These may be considered as stages in the development of the intercellular spaces, which are located, like the areas of less deeply stained substance just mentioned, at the angles formed by the junction

of three or four cells, are enclosed within the apparently split middle lamella, and are commonly angular in form.

Since there is no trace, except in the pine, of a *Zwischensubstanz* whose absorption would leave intercellular spaces, such spaces in the tissues of other plants can be accounted for only by the rounding up and drawing apart at their corners of adjoining cells. This induces a splitting of the middle lamella. The cleft so formed may be temporarily filled by a lax, fluid or semi-fluid substance, a solution, perhaps, of pectic acid or one of its transformation products, but this in time is absorbed into the walls, leaving an empty space. The surfaces of the wall exposed to the so-formed intercellular space may become further modified chemically, and even so softened as to flow enough to form a rounded instead of an angular space; but there is no evidence that such chemical changes ever occur except where the wall is exposed in an intercellular space, and it is more likely that the plasticity here displayed by the pectic acid has characterized it from the time of its deposition, but has not been shown so plainly because of its confinement within comparatively rigid limits.

On the basis of the later investigations respecting the relation of the cell wall to the cell plate, we have seen that the middle layer appearing after the splitting of the cell plate is to be considered as formed by deposition from the split halves of the original plate; the middle lamella of mature tissues would include, then, in addition to possible later deposits, both the layers deposited on the inner surfaces of the daughter plasma membranes. This being the case, we might expect to find in the history of the development of the middle lamella evidences that it consists of two layers. This is just what is found in the case of the intercellular spaces, which are very evidently caused by a split through the center of the middle lamella. If the middle lamella were not of a double nature, we should hardly expect it always to split through the middle, but, in view of its marked difference from the adjoining layers, we should expect that sometimes the whole layer would be pulled to one side or the

other as the cells round up and draw apart at their corners. As noted above, in sections split in cutting, there were also frequent cases where the middle lamella split rather than tore away from the other layers. The splitting in all these cases seems to indicate a weakness of cohesion in the plane between the two layers first deposited by the plasma membranes; and confirmation is thus given of the view advanced by Strasburger and confirmed by Timberlake that the cell plate splits before the new cell wall is laid down, the latter thus having a double nature from the start. We should not overlook the possibility that the very first layer which appears between the daughter plasma membranes is so thin as not to form a noticeable fraction of the thickness of the mature lamella; and that the splitting at a later stage in the middle lamella's history is due to a decomposition of this first thin layer, or that the split is really on one side or the other of it. I have, however, seen no evidence in support of either of these hypotheses.

It cannot be supposed that the middle lamella consists only of the material first deposited from the young plasma membranes. Its thickness in adult cells, and its varying thickness in different tissues of the same plant, *e. g.*, the large vessels and the sclerenchyma of Pteris, at once negative such an assumption. The older view, that it was the cell plate directly metamorphosed into cell wall material, is just as effectively negatived by the same considerations, which should have had more weight with the earlier observers. The middle lamella is rarely difficult of differentiation in adult thick-walled tissues simply by its greater density and less apparent stratification. Moreover, its growth in thickness can often be traced, for it stains continuously from the youngest wall (the thin tangential red lines in the cambium of the rose) to the middle lamella of mature tissues. We must conclude that the middle lamella consists of the layers first deposited by the plasma membranes *plus* a certain amount of material subsequently deposited in contact with these layers, which is generally rich in pectic compounds as compared with still later deposited strata. The middle lamella may vary

considerably in thickness in different tissues, according as a greater or less amount of material rich in pectic substance has been deposited in the cell. That it is not the only part of the cell wall, however, that contains pectic compounds is shown from the taking up by the other layers of the wall, though less freely than by the middle lamella, of pectic acid stains; it is shown also in the pectic layers lining the pits of the pine and the canals in the bast fibers of the rose, and in the innermost thickening layers of the bast of Nerium and rose.

Although the middle lamella usually retains its pectic nature, it undergoes a change by which, in the course of cell development, it loses the power of adapting itself to the varying form of the adjoining cells and becomes fixed and inflexible. The evidence seems satisfactory that this change, as Mangin suggests, is one from pectic acid to insoluble pectates, chiefly the calcium salt. Such a change is indicated by a less deep staining in older tissues, unless they are first treated with acid alcohol. A further transformation appears to take place in the cork cells of Tilia, by which the power of distinctive staining is entirely lost, and the middle lamella cannot be distinguished from the other layers of the suberized wall. The pectic acid here seems to have been partly replaced by suberin. The coloration of the cork walls is purplish, indicating a possible similarity in chemical nature to the later layers of the bast elements in Nerium and rose. In the sclerenchyma and stone cells of Pteris, also, though a middle lamella is plainly present, its staining reactions do not distinguish it from the rest of the wall; yet it can be traced as a continuation of the thin, characteristically-staining walls of the fundamental parenchyma. It is possible that the middle lamella in this case was from the start non-pectic; but it seems more probable that, as secondary thickening proceeded, the pectic acid was replaced or masked by material similar to that deposited to form the other layers. Further light could be thrown upon this question by tracing the development of the wall from the meristem to the mature tissues. The difference in depth of stain between radial and tangential walls in Tilia may indicate that the tangential

walls in this case are not composed of pure pectic acid, or that it exists in them in a somewhat different form. Such a difference in chemical nature might easily occur, since the tangential walls are being laid down anew, while the radial walls are older, and at most are simply added to as the cells increase in number and size. There is no evidence of the existence here of a mass corresponding to Dippel's *Zwischensubstanz*.

The power of the cell to secrete cell wall materials of very different chemical composition at different periods in its history is much greater even than is indicated by Mangin's work. That the compounds stained by ruthenium red are not derived from the decomposition of a previously deposited cellulose wall is proved by the complete staining of the young cambium wall. Still, that there is a possibility of a closer relation than Mangin supposes between cellulose and other cell wall materials on the one hand, and pectic substances on the other, perhaps even involving transformations of material from one class into the other, is to be inferred from the action of ruthenium red upon the walls of the cells surrounding the resin pores in pine. But that the power of secreting pectic compounds is not limited to a single period in the history of the cell is shown by the changes already discussed in the mass of the middle lamella. Since the very youngest and thinnest cambium walls are stained by ruthenium red, and at a later stage the thicker walls are still completely stained, the power of depositing pectic acid must last for a considerable time. That it continues in some cases even beyond the cambium stage is indicated by the variations in the thickness of the middle lamella in older tissues. Pectic substances, though perhaps not in the form of pectic acid, are also secreted at later stages in cell wall development, usually in combination with other materials, as cellulose, callose, and nitrogenous substances. In most of the thickened walls studied, evidence of the presence of pectic substances was found in the secondary thickening layers. The general rule, that pectic compounds are deposited in the cell wall early in the life of the cell, and that layers deposited later are predominantly non-pectic, is seen to have exceptions

in the case of the innermost strata of the bast fibers of Nerium and rose, and in the lining of the canals of the rose and of the pits of the pine. In these cases we have the deposition first of a pectic layer, then of non-pectic or mixed layers, and then again of a pectic layer. The purplish tinge imparted to the bast thickening layers just mentioned by ruthenium red indicates that these layers consist of some pectic substance or substances, other than pectic acid, at least in the form in which it exists in the cambium walls and the middle lamellae of other tissues.

Whether the period during which the cell generally deposits pectic acid, and thus forms the middle lamella, marks any special stage in its development which can be sharply distinguished from subsequent periods in its history is not certain. The evidence at present available seems to indicate that the pectic layer continues to increase in thickness about as long as the cambial cell is increasing in size. It is possible that the attainment by the cell of its adult size marks the limit of the growth of the middle lamella. In this case we might say that pectic acid is deposited so long as the metabolic processes of the cell result in a *plus* which is expressed in cell growth, but that later, when a metabolic equilibrium has been established, or when the excess of food is stored instead of being used for growth, or when the protoplast degenerates, a predominance of other cell-wall materials is deposited. The evidence for such a view, however, is far from complete. It must be remembered, too, that in the cambium cells the distinction between middle lamella and inner non-pectic layers is very early present, and that the middle lamella continues to increase in thickness after it is separated from the protoplast by the non-pectic strata deposited later. The middle lamella is not to be considered as consisting merely of the wall layers early deposited by the protoplast, without undergoing any later modification. It is quite possible that the pectic acid which characterizes it may in part be secondarily formed or deposited by infiltration through non-pectic layers which separate it from the protoplast. Some such hypothesis as this is necessary to account for its increase in

thickness during the later stages of cambial growth. Of interest upon this point is the fact noted by Mangin that, although in the meristem of some of the plant tissues studied by him no middle pectic layer could be detected by staining, yet, upon a treatment known in other cases to dissolve pectic acid, the cells are dissociated. My study of very young rose shoots, though not carried back far enough to dispute Mangin's results, yet shows that, at a point where the tissues have become very slightly differentiated from the meristem, the cambium walls show strongly by their affinity for ruthenium red their pectic nature.

CONCLUSIONS.

The facts that have been cited seem to me to show conclusively that the middle lamella is not merely the partition wall first laid down, either as a single or a double layer, by the plasma membranes. Nor is it, on the other hand, merely an intercellular substance of cement, a means for binding the cells together, as Mangin holds. Individual cells are not separated from one another, either in their formation or their later development, and no reason appears why an intercellular cement should be secreted. There is, however, abundant reason why, in woody tissues especially, there should be during cell growth a plastic region in the cell wall, which should be in a measure adaptable to the changing size and form of the protoplast itself, and to the firm, resistent layers whose form must correspond to that of the protoplast at the time of their deposition. The middle lamella is, therefore, a wall layer with a complicated history, undergoing after its first appearance changes in form, increase, and probably at times decrease in mass, and changes in chemical composition; its history, too, is not identical in different tissues. There can be no doubt that, in the higher plants studied, the young cambium walls are included in, and form the basis of, the middle lamella of the older tissues. These may at first include, at least in the pine, an amorphous *Zwischensubstanz*, which occupies a cleft formed by the splitting of the young radial walls. Quite early in the history of the cambium cells, a non-pectic layer

appears within the first-formed pectic stratum; but the latter, now properly a middle lamella, continues to increase in thickness even after the appearance of the non-pectic layer. The substance of the middle lamella, now, is not rigid, but is more or less plastic, or even, as Dippel believes, soluble under certain conditions of development. Its solubility may lead at times to its partial absorption by the rest of the wall. More often, probably, its mass is increased by further secretion of pectic material from the adjoining cells into the spaces formed by their rounding up. The plastic nature of the whole layer allows the modification of its shape, also, as the cells round up and as more resistent layers are deposited against it, leading to its being massed at the corners and pressed out to a thinner layer at the sides. The pressure may be in certain cases so great at the corners as to increase the density at those places; but in general, as the cells round up and draw away from the corners, the pressure there is relaxed, and, if new material is not deposited, the substance becomes less dense, perhaps swelling by the absorption of water. Or it may be that already the middle lamella has split, and the cleft has been filled by the exudation into it of a pectic fluid, which is later to be reabsorbed. At any rate, sooner or later the tension incident to the rounding-up process brings about a split in the middle lamella, and an intercellular space is formed. In case a *Zwischensubstanz* was present, a splitting at this time is unnecessary, and the intercellular space results from the absorption of this substance. If the substance of the middle lamella is sufficiently rigid, the intercellular space remains angular; but if the material still retains some plasticity, or if it undergoes a chemical change upon its exposure at the newly formed surface, the angles may be rounded, or may disappear entirely, leaving a circular or elliptical space. The chemical changes effected in the middle lamella after the cell has attained its final form consist in the conversion of pectic acid into insoluble pectates, or even, as in the cork cells of Tilia, its replacement by entirely different substances.

It seems to me plain that the term "middle lamella,"

indicating merely the position of the layer in question, is preferable to either "cement" or "intercellular substance." The latter term, arising from a totally false conception as to the origin of cells, should certainly be dropped. There is also no evidence that the cell wall layers which, taken together, form the middle lamella have any significance as a cement for binding the cells together, and so it is inappropriate to apply to them a name suggesting any such function.

The fact of the origin of the middle lamella as a double layer deposited on the surfaces of two plasma membranes, and the fact that in the formation of intercellular spaces it always splits in a median plane, must always be borne in mind. This constant plane of cleavage may be called the primary cleavage plane, or, perhaps, the primary cleft when it is spoken of with reference to the space between the daughter plasma membranes in which the cell wall material is deposited.

I would distinguish cambial walls as the walls of cells yet capable of division, and primary cell walls as those layers added during the growth of the cambium cell into a full-sized wood or bast element. Secondary thickening would include the strata deposited in the subsequent history of such cells. This secondary thickening may be subdivided further where evidence exists that its deposition has been interrupted and subsequently resumed; we should then have tertiary thickening, and so on.

For the adult condition of thickened cell walls, the following terminology might be proposed as most exact from the standpoint of our present knowledge:

The boundary plane between adjacent cells, commonly invisible except where indicated by intercellular spaces, would be, following the suggestion already made, the primary cleavage plane. Including this on both sides, we should have the apparently homogeneous middle lamella, including the cambial walls and more or less of the primary cell walls, according to the proportional pectic acid content of the latter. Then would come those layers of the primary wall, if there be such, which are predominantly non-pectic; and lastly the layers of secondary and

perhaps tertiary thickening formed in the metamorphosis of the adult cell into its permanent condition as the element of a tissue.

The investigations here discussed were undertaken at the suggestion of Professor R. A. Harper, and have been carried on under his direction. All the results accomplished are due in the highest degree to his suggestions and assistance.

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STRUCTURAL STUDIES ON SOUTHWESTERN CACTACEAE.

CARLETON E. PRESTON.

(WITH NINE FIGURES)

THAT the Cactaceae, a family in every respect appearing to be of recent origin, are unique in the extent of their adaptive modifications to arid conditions is a commonplace. Not only does this adaptation affect the external parts as gross structures, but in still greater measure does it work changes upon the minute anatomy by modifying the histological elements. These internal changes, keeping pace with and supplementing the external, raise this family to the first rank among the desert plants. A critical study of the minute anatomy from the standpoint of ecology, therefore, should be most fruitful. Moreover, since the family is one of late origin, in which definite groups have so far not crystallized out, but between which nearly all connecting links are present, a general comparative examination of internal structure should afford at least some further clue as to the lines upon which the various groups have developed or are still developing, adding to or confirming the evidence already obtained from the study of external characters.

Viewed from the standpoint of the systematist, this paper, being a study of the general histology of eight southwestern forms, can by itself be of little value, serving only as an addition to the number of species already critically examined. Although in collecting the material for study it was my intention to choose such forms as could be considered typical, I found, as work went on, that too much variation occurs to admit of their use in this way except upon the broadest lines. In order to do complete and satisfactory systematic work on the basis of structural characters, evidently one would have to compare, not only all the genera within the family, but at least the larger subdivisions and

the doubtful species in each genus. The single genus Cereus contains a multiplicity of forms, the structures of which must be very diverse. How are these related, and what are their several connections with the other genera? Does the gradual change in internal structure agree with the external and strengthen the validity of the various systematic groups as now recognized? Schumann[1] has pointed out that the original ancestor of the Cactaceae was probably something like Peireskia. Ganong[2] has followed up and confirmed the relation between this genus and Opuntia, chiefly on the basis of external characters. What are the steps by which the corresponding internal structure has changed? Schumann[1] has hinted rather strongly at the connecting links between Opuntia and Cereus; anatomical studies may give more definite information upon this point.

This paper seeks to impress the importance of careful structural study to help out the ordinary work on external characters, to point out the general trend of evolution exhibited, and to interpret certain anatomical conditions upon a physiological basis.

As regards environmental adaptation, next to diminution of surface and other modifications to lessen transpiration, the storage of water is most important. The turgor exhibited in many of the more succulent forms is surprising. Especially is this the case in the younger portions of the arborescent species, and throughout the interior of those which are low and cespitose, where for a long time turgor alone is sufficient to sustain all the weight placed upon it—in other words, to preserve the rigidity of the plant. Hence there is no necessity for a strong vascular system; in fact, its room is more valuable than its presence, and we find in general that the vascular system is of late development, and in low forms almost never reaches a high state of perfection. The component elements also are oftenest of a kind generally considered primitive, showing an apparent retrogression, but one which in many cases is complicated by the

[1] Gesammtbeschreibung der Kakteen. 1897.

[2] Beiträge zur Kenntniss der Morphologie und Biologie der Kakteen. Flora 79: 49–86. 1894.

addition of adaptive modifications. This is true particularly in the first form examined, *Cereus Fendleri*, of cespitose habit, and one of the most succulent. Upon cutting a shoot transversely one will notice that the bundles recede into the tissue for some distance, both in the severed portion and in that remaining on the plant, while the parenchymatous tissue still remains turgid. It is thus easily demonstrable that the bundles are normally under great tension. The mechanical arrangement by which such elasticity is rendered possible will be found in the structure of the xylem. Another point of interest, probably of value upon systematic grounds, is the behavior of the bundles in the matter of secondary medullary ray formation, development of interfascicular cambium, and anastomosis.

Before considering the various forms separately, there are certain points of structure which can best be mentioned in general. Schumann, both in his monograph and in Engler and Prantl's *Pflanzenfamilien*, gives an idea of the ordinary structure of the group. A condensed account is also given by Ganong[3] which I take the liberty to quote as a basis for the following discussion.

> As to the tissues, it is enough here to say that the characteristic xerophilous appearances are a strong cuticle, thick epidermis, perfect cork, sunken stomata, collenchymatous hypoderma, deep palisade layers, great development of pith and cortex, which consist of large, round, splendidly-pitted water-storing cells, often containing mucilage; a fibrovascular system, in general simple in its make-up, lacking annual rings, composed as to its xylem of strongly-ringed and spiraled tracheids, which are often collected into gland-like masses, the whole system conforming closely to the external form and following its morphological changes.

Regarding the character of sunken stomata and their relation to hypoderma slight comment is necessary. The stomata are not sunken in the sense of having deep-lying guard cells; on the contrary, they are well upon the surface, as Schumann notes. Whatever vestibule there may be is of a different nature. With the increase in thickness of hypoderma an interval is produced

[3] Recent problems in the anatomy, morphology, and biology of the Cactaceae. Bot. Gaz. **20**: 130. 1895.

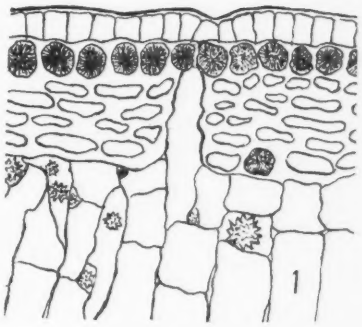
between the assimilative tissue and the guard cells. Through this collenchymatous tissue run canals connecting the two; these canals must at all times be filled with water vapor, and probably act in a much more effective manner than would an equally large external vestibule. The hypoderma is of varying thickness, of one cell layer only in some species of Mamillaria, of seven to ten in certain other forms, as in *Cereus giganteus*. There appears to be no difference in the character of the cells in the various groups; the walls are irregularly thickened, the thin portions allowing osmotic transfer of materials between the cells, and from them to the epidermis. Portions of hypoderma are shown in *figs. 1–3*.

With respect to assimilative tissue, Ganong speaks of deep palisade layers. Schumann, on the other hand, says that the cells are not in palisade form, but spherical. In point of fact, there seems to be considerable variation in this regard. In no form which I have examined have I found an assimilative tissue of absolutely spherical unmodified cells. There has always been an elongation perpendicular to the surface, sometimes slight, as in *Opuntia leptocaulis*, in other cases very well-marked, as in *Echinocactus Wislizeni*. There is also a general compacting of the tissue in this region. These elongated cells extend inward in the form of more or less definite chains or filaments, grading rather slowly into the ordinary parenchyma tissue, which in many cases contains chlorophyll in some quantity, in others is colorless. In the latter instance the chlorophyll, as noted with the naked eye, seems confined to a well-marked rind.

It is perhaps only fair to suggest that environment may have a considerable influence upon the development of the palisade form in this family, as well as in those which possess functional leaves. The classical works of Stahl, Pick, and other investigators leave little question but that the elongation of the assimilative tissue, at least in some degree, is in direct response to the stimulus of light. The habitat of the plants here described and contrasted is one marked for its great light intensity, which, as I have found from a rather comprehensive examination of foliar

structures, leaves its imprint upon the arrangement and development of assimilative tissue in almost all cases. Schumann's statement that the assimilative cells are spherical may be true of the plants when a general average is taken, including epiphytic types, and others growing under entirely different light conditions. Possibly also the effects of artificial cultivation may have some place in his results.

The medulla in many cases is of thick-walled and pitted cells, as Ganong describes, but this is by no means universal, often varying within the limits of a genus as now established. The cells are often exceedingly thin-walled and have no need of pits to maintain connection between them. The presence in this tissue of mucilage, crystals, etc., is likewise variable with the species, and needs separate discussion. Not only do the crystals vary in distribution to a wide extent, but also in relative frequency, some species being far richer in this deposit than others of the same habitat, a fact which, it seems to me, can only be referred back to the varied selective power of roots for chemical solutions.



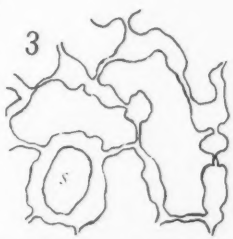


FIG. 1.—Epidermis and hypoderma of *Opuntia arbuscula;* opening of stoma does not show, owing to slightly oblique character of section.

FIG. 2.—Epidermis and hypoderma of *Cereus Fendleri* in section, showing stoma.

FIG. 3.—Hypoderma of *Cereus Fendleri*, seen from inside, showing character of walls and opening of stoma canal (*s*).

The particular value of Ganong's terse and concise description of the fibrovascular system lies in the last clause, "the whole system conforming closely to the external form, and following its morphological changes." Upon this fact depends the usefulness of internal structure as evidence of systematic value, a point which this writer thus recognizes, and one which I feel that I cannot too strongly insist upon. Schumann gives a slightly fuller account of the vascular system, noting the presence of annular and spiraled ducts, *especially in young bundles*, and their subsequent replacement by the various tracheids. This is also the statement of the fact that a regression toward more primitive conditions is taking place, by the substitution of elements of a less highly developed character. In this particular especially considerable variation is shown, offering, together with the methods of bundle-branching and anastomosis, a very fruitful field for research from a systematic point of view. Annual or periodic rings are not always absent, as will be shown; in fact, in one form I have examined they are peculiarly marked.

The work presented in this paper was done on fresh material collected in the vicinity of Tucson, Ariz., during December 1900, and examined immediately at that place. Opportunity was thus given to study the plants in their natural habitat, a very valuable advantage in dealing with physiological phenomena.

CEREUS FENDLERI Engelm.—The hypoderma is comparatively thin, of about three layers of cells; the cuticle is thick as usual; there are no crystals in the epidermis, only an occasional one occurring in the deeper cells of the hypoderma. The assimilative cells are large and fairly elongated, those nearest the surface containing the most chlorophyll, those toward the center grading into a very thin-walled parenchyma also slightly chlorophyllous, and filled with an intensely stringy and slimy mucilage, which, by taking stains readily and by preventing dehydration, renders any delicate manipulation of sections upon fresh tissues out of the question. It is by this means that the plant is able to hold water so persistently. In the parenchyma are imbedded the bundles, slightly separated from each other in most cases,

but with some anastomosing. The lateral branches, which apparently start for the tubercles, break up and form a network through the tissue. In this species the bundles appear to start in a definite circle in cross section, in contrast to the undulate line in which they occur in certain other forms. There seems to be no interfascicular cambium, the bundles in their exceedingly slow growth apparently pushing their way outward into the succulent tissue, compressing it about them, and taking up the room it formerly occupied, instead of the growth of the medullary tissue keeping pace with the increase of the bundles. The development of phloem is exceedingly slight, consisting wholly of sieve tubes and companion cells, with no trace of thick-walled elements.

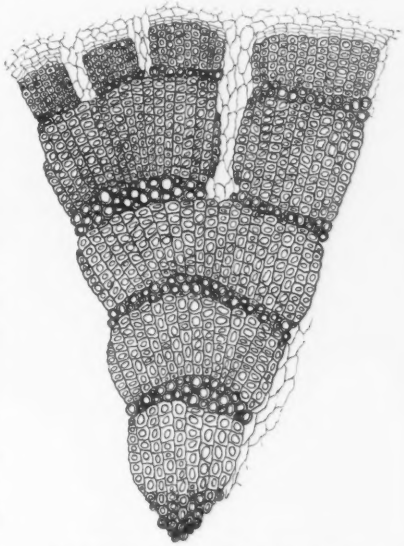


FIG. 4.—Xylem portion of stem bundle in *Cereus Fendleri*.

Of the xylem more needs to be said. A bundle is represented in *fig. 4*. The elements which compose it are but two, spiraled ducts and tracheids with a peculiar flattened spiral, the latter being by far the more abundant. These alternate in time of development, showing what appear to be periodic changes. The very earliest portions of the xylem are composed of the ordinary spiraled ducts, irregular in size and position, and thereafter a small layer of these cells appears at what is probably the beginning of each season's growth. The flat spiraled tracheids, an element already mentioned by Ganong and Schumann, form in definite rows, each apparently the product of a single cambium cell, which, after cutting off the few irregular segments to form the spiraled ducts, apparently undergoes no more division by radial

walls during the season. All the broadening of the bundles, therefore, occurs at the beginning of a period of growth. Likewise, when a branching is to occur, the resulting medullary ray appears at the beginning of the season, as shown in the drawing.

It seems likely that the flattening of the spiral in the tracheids, which compose most of the xylem, is what gives the elasticity so prominent in this bundle. Such a flattening inwards would allow a far greater stretching of the spiral than would be possible in the commoner round type. A fact which strengthens this assumption is that the walls of the tracheids in a preparation will be seen to be wrinkled between the spirals (*fig. 5*). In the living condition those were in all probability perfectly straight, and the turns of the spiral at a greater distance apart.

FIG. 5.—Portion of tracheid of flattened spiral type from xylem of stem bundle in *Cereus Fendleri*.

Another point which can be deduced safely from an examination of the figure is with respect to periodicity of growth. From the comparison with the amounts of earlier years, it will be seen that the xylem for the season was about half formed at the time of collection of material. As this took place in the latter part of December, it seems probable that the season of growth in the southwest is limited by the summer drouth and intense heat, rather than by the winter cold.

The branching of bundles and formation of secondary medullary rays in the stem is not of common occurrence in the Cactaceae, so far as my investigation goes. In most of the forms to be described a different state of affairs will be noted. The bundles of the root, however, branch in the ordinary manner in all the species I have examined.

The absorptive root[4] of *Cereus Fendleri* has a cork four to six cell layers in thickness. Successive layers of this kind are formed, with parenchymatous intervals, so that the bark is being constantly shed in flaky fragments, and is with equal rapidity replaced. The cork cambium lies a short distance outside the

[4] BOT. GAZ. **30**: 348. 1900.

secondary phloem of the bundles. This phloem is composed almost wholly of sieve tubes which, when examined January 1, had no callus visible. Lateral plates occur in abundance, in addition to those at the ends of the cells.

The xylem has but two elements, at least in the secondary portions. The more prominent, and by far the more abundant of these, is the tracheid, with ellipsoidal pits in more or less regular lines, an element which, from the shape of the pits, hardly coincides with the ordinary scalariform vessel, as seen in Pteris, for instance, but which, for convenience, I shall here designate by that term. Though the separate cells are comparatively short, there is often a continuous passage through several (a transition stage toward the true trachea), the walls between the adjacent cells being partially dissolved, and represented by nothing more than a thick and distinct ring. The other element is the obliquely pitted fiber tracheid, which apparently supplants the ordinary wood fiber in the majority of the Cactaceae.[5] The medullary tissue is but little compressed in the rays, giving a rather loose structure to the root as a whole. Crystals here, as also throughout the entire plant, are very few in comparison to their number in all the other species examined, occurring in the various parts of the parenchyma tissue, always in the aggregate form.

The anchoring root differs from the absorptive chiefly in the relative numbers of fibrous and vascular elements, as stated in a previous paper,[6] in which the phrase "wood cells" was used by mistake for "fiber tracheids." There is also a slight difference in the amount of sieve tissue developed in the phloem, the greater amount belonging naturally to the absorptive root. In species where the two systems are not clearly differentiated, the elements are more nearly equal as regards amount of space occupied by each. As a rule, the only difference between the two roots is one of proportion of elements, not of their variety.

[5] For a drawing of this element see Strasburger's *Text-book of Botany*, translated by Porter, *fig. 143 ft.*

[6] Bot. Gaz. **30**: 348. 1900.

The absorptive root, therefore, will be the only one taken under consideration in discussing the remaining forms, except in one or two special cases. The difference between the two is hardly as well marked in *Cereus Fendleri* as in *Opuntia fulgida*, to be mentioned later.

ECHINOCACTUS WISLIZENI Engelm.—The epidermis and hypoderma are without crystals, the latter of six or seven layers of cells. The assimilative tissue is compact, with cells much elongated perpendicular to the surface, even in the sinuses between the ridges. The young parts do not develop chlorophyll in the sinuses until the ridges of tubercles, at first close-pressed together, become separated. The chlorophyll in this form is confined to a definite rind of palisade-like cells, which on the inside changes somewhat abruptly to a colorless parenchyma, rather thick-walled and fitted with a splendid system of pits and openings. Within this tissue are occasional crystals. Mucilage does not seem to be abundant in this species, but its place is taken by a watery solution, especially prominent in parts still growing, which upon long exposure to the air causes the tissue to assume a pinkish tinge. The medullary tissue about the bundles contains a fairly large quantity of starch, greatest in amount and widest in distribution in the young portions.

The bundles, as seen in cross section in a young stage, are not in a perfect circle, but rather in a zigzag line (*fig. 6*, diagram), with alternate points and depressions. There seems to be no branching or secondary medullary ray formation, and but little increase in the width of the original bundles. The zigzag line does not appear to be entirely the result of bundles passing out of the tubercles, for nearly all of the bundles in section appear to be cut directly across when all are cut in the same plane. They anastomose to a considerable extent, and, taking as centers the bundle at the base of each inner depression, join together with it to form a complex wood structure. The direction of growth of the side bundles is slightly inclined toward the central one, bringing the xylem of the whole group into

close contact at an early stage. The phloem portions, however, remain distinct for each bundle. By this slight divergence, considerable medullary connection is left between the pith and the thick layer of storage tissue outside the poorly developed bundle cylinder, a far greater connection than could occur with the usual distribution, thickening, and branching of bundles seen in dicotyledonous stems. This peculiar grouping will be noted

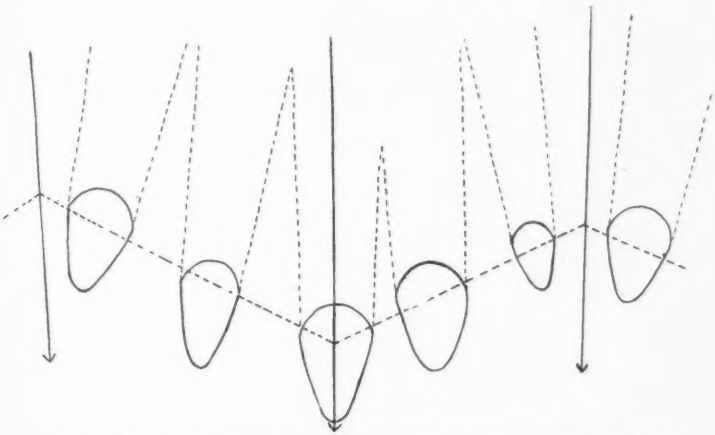


FIG. 6.—Diagram of position of bundles in *Echinocactus Wislizeni* and others.

in a far greater degree of perfection in *Cereus giganteus*, next to be described. Here, as there, there seems to be no anastomosis of these groups of bundles, the incipient wooden ribs. In Opuntia, on the contrary, where this anastomosis of bundles likewise occurs to some degree, a still further reticulation is found, in that the groups as units anastomose.

As regards more minute structure in *E. Wislizeni*, the phloem is composed mainly of sieve vessels, the outer of which, together with the adjacent parenchyma, are composed and rendered sclerenchymatous, forming a sheath, here separate for each individual bundle. The xylem, at least in later portions, is composed entirely of spiraled tracheids of uniform size, but not in definite rows, the spiral also being of the ordinary type. A

certain amount of elasticity of bundle occurs here, but nothing like that in *C. Fendleri*. The enormous amount of thick-walled parenchymatous storage tissues seems to have more effect in giving firmness to the internal structure than does turgor. The bundles have almost no supporting function, and are never well developed, appearing, in the widest part of a fair sized specimen, only 1^{cm} in thickness of xylem. The branches passing out to the tubercles or ridges divide through the tissues as in *C. Fendleri*.

The absorptive root contains numerous small crystals in the rays, more in proportion than appear in the stem. The chief xylem element is the scalariform tracheid; fiber tracheids also occur. In the anchoring root, as before, the proportions are reversed. The phloem is mainly composed of sieve tubes, but has a slight sclerenchymatous compressed sheath, a character which will be noted as fairly constant in both stem and root through the majority of forms.

CEREUS GIGANTEUS Engelm.—The epidermis and hypoderma are both without crystals, the latter being exceedingly thick, of twelve to fourteen layers of cells. The assimilative tissue is of the usual elongated cells, the filamentous arrangement of which is extremely well marked, to such a degree even that the filaments in a preparation can be separated easily. Chlorophyll extends deep into the tissue, and the elongated character of cell is found for nearly 20^{mm}. The medullary tissue is thick-walled and pitted, containing starch in the immediate vicinity of the bundles. Mucilage, though present in quantity sufficient to hinder the staining of sections, is not very thick. There is, however, especially in the younger parts, a watery solution apparently identical with that seen in *E. Wislizeni*, but much stronger, causing the tissue upon exposure to turn almost immediately from white to pinkish, and then to a dark purple.

In bundle formation, the method described for *E. Wislizeni* above is carried to greater perfection. Here not only do the bundles join their xylem portions, but all those of a single rib are surrounded by one sheath, formed gradually. In a young stage it may be seen outside each separate bundle, later two or

more bundles are embraced by a single sheath, and this process apparently continues until all the bundles of the rib are thus bound together. This sheath is not the ordinary one of compressed sclerenchyma so commonly found next the sieve tubes. This outer one seems to be a special structure, composed of the obliquely pitted fiber tracheid so often mentioned, which here inclines more than ordinarily to the tracheid form, with wide lumen and with the extremities of the cells scarcely pointed. The individual cells are much larger than those of the same nature found in the xylem. The ribs alternate with the ridges of tubercles, both undergoing more or less branching. A full-grown rib, according to measurements taken from dead skeletons, seldom exceeds 3^{cm} in thickness. The phloem has the usual development of sieve tubes; the xylem is made up almost wholly of scalariform tracheids, an element so far noted only in the root. Besides these, there also occur fiber tracheids, undoubtedly in increasing proportion as the size of the rib and the corresponding function as a support increases.

The absorptive root differs from the others described in having in the phloem an abundance of fiber tracheids, forming a wedge pointing outward, starting just outside the sieve tubes of each bundle. These cells are also numerous in the xylem, together with the scalariform tracheids. Crystals are common in the cortex as well as in that of the stem, and are large and closely aggregate. They do not seem to be entirely homogeneous, but take some foreign substance as a nucleus. At times they appear to grow by concretion, showing when viewed in optical section a series of concentric circles.

The necessity for a fibrous strengthening in the absorptive root of this large form can best be understood by examining the method of growth. In starting as a seedling, the plant begins life with a base slightly sunken in the ground, but far narrower than the heavy column which it is to support. Therefore, as this column develops, the whole becomes very top-heavy, and would certainly fall were slightly greater surface exposed to the wind. The anchoring root, at this time well developed, holds

it firmly to the ground, but only to a slight degree prevents a swaying movement, easily induced, even in plants 1.5^{m} high, by comparatively little force. Hence to the large lateral roots, developed from the sunken base at a later period, is given the function of steadying the whole plant, in addition to their primary work of absorption.

MAMILLARIA GRAHAMI Engelm. — The epidermis is free from crystals, but the one-layered hypoderma contains them in almost all its cells. The assimilative tissue is of small cells elongated, as usual, perpendicular to the surface. The colorless medullary portion is of thick-walled cells pitted as in *E. Wislizeni* and *C. giganteus*, and grades into the assimilative tissue. The lateral bundles can here be traced out into the tubercles. The stem bundles appear to start in a more or less wavy line, and to suffer fusion to some extent. There is comparatively little branching, but secondary medullary rays are occasionally found, and there is also a growth of the bundles in width, so that finally a rather compact cylinder is formed. As regards branching and internal structure of bundle, this species approaches *C. Fendleri* nearer than any of the others. The xylem is composed of alternating portions of spiraled ducts and tracheids of the flattened-spiral type, the latter being the more prominent. The phloem has a slight compressed sheath of sclerenchyma. Sieve tubes appear rather poorly developed, a thick-walled, somewhat elongated parenchyma taking up most of the region of the phloem.

In this small species there is apparently no distinction of anchoring and absorptive root systems. The bundle cylinder is compact, the pith practically absent, and the medullary rays reduced generally to a single cell layer. The phloem is slight and of sieve tubes. Outside the phloem, filling the small space between it and the cork cambium, is a parenchyma tissue of large rather thick-walled cells. The xylem consists chiefly of spiraled tracheids, with some amount of extra reticulation, and of smaller ringed ducts, the rings flattened inward, sometimes also bound together by extra spirals or reticulations. Crystals occur but

seldom in the root, but are present in some quantity in the cortex and medulla of the stem.

OPUNTIA PHAEACANTHA Engelm.—The hypoderma is rather thick, of six to eight layers, the outer one compactly filled with crystals, which occur also in great numbers throughout the assimilative and medullary tissues, clustering more or less about the bundles, but especially prominent in and about large duct-like spaces just exterior to certain of the bundles in both stem and root (Schumann's *Krystalschläuche*). Mucilage is fairly abundant. The assimilative cells are somewhat elongated, perhaps less so than in other forms so far recorded. Chlorophyll is deepest in the outer layers, but present as well in almost all parts of the deeper thin-walled parenchyma. The stem bundles form a loose reticulated network, branching early, especially on the flat surfaces of the stem, forming very wide secondary medullary rays. The phloem has the usual development with a sclerenchyma sheath. The xylem in early parts consists almost entirely of smaller annular ducts with flattened rings, but later forms spiraled tracheids and a large amount of obliquely pitted fiber tracheids.

In the root especially are seen the *Krystalschläuche* in a high state of development, with a diameter of almost 1^{mm}. The crystals seem to be deposited mainly along the edges of the duct, which may serve also to some extent for the transfer of mucilage. The phloem of the bundle has a slight sheath; the xylem is composed of large and small scalariform tracheids and the usual fiber tracheids.

OPUNTIA FULGIDA Engelm.—The epidermis contains a deposit of crystals, a small quantity of which also occurs in the relatively thin hypoderma. The assimilative tissue is of cylindrical cells in lines, grading in the interior into large thin-walled parenchyma, also chlorophyllous. Mucilage is exceedingly abundant, and the ducts for its accommodation and transfer are both large and numerous. Large crystals, loosely aggregated, occur in small numbers throughout the tissue.

The bundles here remain very small through several joints,

later becoming fused into a reticulated skeleton, the separate bundles of a single strand also anastomosing. The wavy line formation is seen to a slight extent in these Cylindropuntias also; there is apparently no branching, and little increase in width of bundle. The phloem presents no unusual characters; the xylem is composed of annular ducts, with flat rings at regular intervals; later on there occur also spiral tracheids, with steps grading to the true trachea.

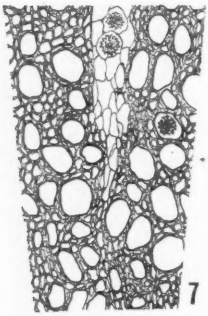


FIG. 7.—Cross section of portion of xylem from absorptive root of *Opuntia fulgida.*

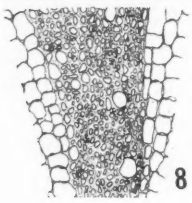


FIG. 8.—Cross section of portion of xylem from anchoring root of *Opuntia fulgida.*

In an earlier article the roots of this species have been referred to as anchoring and absorptive. Portions of the xylem of each are figured here to show the difference in texture (*figs. 7, 8*). In the absorptive roots the elements are wider, more regular, and less thickened. The phloem differs but slightly in the extent of development of sieve tubes; the xylem differs as usual in proportions of elements, consisting in both of scalariform tracheids and fiber tracheids. From the prominence of the latter, both here and elsewhere, it would appear that the main function, if not the only one, which this element performs in the Cactaceae is one of support.

OPUNTIA ARBUSCULA Engelm.—The hypoderma is fairly thick, with a compact deposit of crystals in the outermost layer. The assimilative tissue is compact, the cells being small and greatly elongated in regions near the surface. The chlorophyll extends through the thin-walled parenchyma as well. Crystals are very abundant throughout, varying considerably in size and compactness of aggregation. The stem is very turgid and mucilaginous, kept rigid by turgor for some time, with late bundle development. The bundles finally form the same kind of reticulated skeleton seen in *O. fulgida.* The phloem is as usual; the

xylem shows an occasional ringed duct, apparently an early element, but is principally composed of scalariform and fiber tracheids. Starch is present in considerable quantity through the stem.

The cortical parenchyma of the absorptive root is thickened in many places for storage purposes. The cells are filled with a thick mucilage, which aids in holding the water. A considerable deposit of starch, as well as of the regular calcium oxalate crystals, occurs in the medullary rays, but not at all in the cortex, an apparent differentiation of regions for various reserve substances. The phloem is of ordinary character, with a slightly developed sheath; the xylem is composed mainly of scalariform tracheids of various sizes, but contains a few fiber tracheids as well. A rather peculiar phenomenon is presented by the convergence of the cortical tissue toward the center of the phloem. Whether this was to hasten transfer of materials between cortex and phloem in young stages, before the sheath was thickened, in a way analogous to the clustering of palisade about the leaf bundles, which so commonly occurs, is a question which at once suggests itself, but upon which I am able to throw no light at present (*fig. 9*).

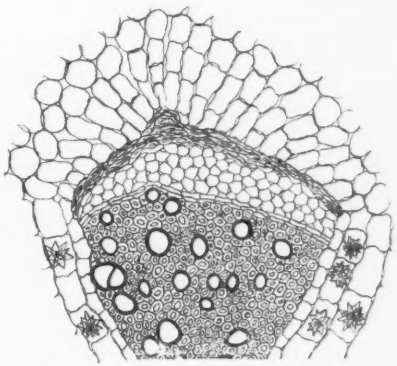


FIG. 9.—Portion of bundle from root of *Opuntia arbuscula*.

OPUNTIA LEPTOCAULIS DC.—The epidermis contains crystals in cells specially enlarged to receive them. The hypoderma is of four or five layers, and the assimilative tissue is elongated very slightly. The parenchyma of pith and cortex is nearly colorless, thin-walled, and contains considerable starch, evidently of the translocation variety. This species is not so succulent as the majority, and has an earlier development of the bundles. A point in which it differs from all the rest examined is in its

possession of a well-marked interfascicular cambium in the stem, allowing the medullary tissue to increase in proportion to the growth of the bundle, instead of having the bundle compress and supersede it, as is apparently the condition in the others. That even here this cambium is not so active as that of the bundle seems to be demonstrated by the fact that outside the phloem the sclerenchyma sheath still appears compressed, though to a less degree. The xylem is composed mainly of fiber tracheids, intermingled with which are scattering spiraled ducts. The ordinary Opuntia reticulation occurs together with the finer anastomosis of the bundles of a single strand. Between these closely connected unbranched bundles the medullary rays develop very thick walls, apparently lignified, and are so filled with a deposit of starch that in section they appear much more opaque than does the rather close-grained wood on each side of them. Mucilage occurs, but not so abundantly as in the majority of cases, and crystals of rather small size are scattered through all the parenchyma tissue.

The root does not materially differ from those already described. The cortical parenchyma is slightly thickened, and the xylem is composed of the scalariform and fibrous types of tracheids.

SUMMARY AND CONCLUSION.—Several inferences may be drawn from the foregoing descriptions. In the first place, it will be seen that the roots, as regards branching and elements concerned, apparently undergo but slight variation, being composed, in almost all cases, of fiber tracheids and scalariform tracheids in the xylem, and of sieve tubes only in the phloem. To this general rule, as regards xylem portions, *Mamillaria Grahami* is the only exception among the forms here compared, and even in this the tracheid appears in greater prominence than the true duct. This exception, however, is enough to suggest that a more comprehensive examination will reveal more of a similar nature, and also perhaps disclose some links in the evolutionary chain between this and and the ordinary type.

In the stem, on the other hand, there is a great range of

structural deviations, which seem to take place along definite lines and by definite steps. This variation extends to bundle branching and further reticulation, to the extent of succulence, character of parenchyma of pith and cortex, development of mucilage, and even to the kinds of elements composing the xylem of the bundle. In the phloem alone there is little change. The practically universal substitution of fiber tracheids for wood fibers, and the equally common appearance of tracheid elements in place of true ducts, except in early growth, points certainly to a general regression. The fiber tracheid, moreover, is variable in itself. In most cases it is quite sharply pointed and thick-walled, with a lumen, though broader than that of an ordinary wood fiber, still rather narrow. In a few instances, however, this same element loses its pointed character, widens its lumen still more, and except for its thick walls and oblique pits, scattered and exceedingly small, closely resembles an ordinary tracheid. This is very well shown in *Cereus giganteus*, a rather anomalous example, however, since here the fiber tracheid supplants a bast fiber instead of a wood fiber, and does not undergo an equal enlargement in the xylem portions of the bundle.

Crystal distribution and various storage peculiarities should be looked upon more as specific differences, I believe; likewise the thickness of hypoderma.

A word or two may be said of *Opuntia leptocaulis*. The early development of the bundles, the smaller degree of succulence, the persistent character of the leaves, at least in some varieties, as noted by Coulter,[7] the very slight elongation of the assimilative tissue, and above all the persistence of the interfascicular cambium, all tend to place this form in structure much nearer the ordinary dicotyledonous stem than any of the others examined. It seems safe to conclude that this species comes lower in the evolutionary line of adaptive modification than the rest of the Opuntias here mentioned, and may be considered nearer the primitive form.

[7] Contrib. U. S. Nat. Herb. 3: 456.

Perhaps the most striking results of any to be found here from the systematic point of view will be obtained by a comparison of the first three forms. *Cereus Fendleri* and *Echinocactus Wislizeni* are entirely dissimilar as regards character of medullary tissue, mucilaginous contents, firmness of interior structure, and especially in respect to arrangement and branching of bundles. The third form, *Cereus giganteus*, corresponds in all the above mentioned peculiarities, not with the other Cereus, as would be expected, but with the Echinocactus. So far as I can tell from plates,[8] this closer resemblance to Echinocactus is carried out equally well in external characters, especially in those of the fruit, but to some degree at least in those of the spines and areolae (the last comparisons taken from living material). I have too little systematic knowledge of this group to contest the position of the species in classification, and can simply point to the fact that the two Cerei here mentioned belong at least to two different evolutionary branches within the genus, from one of which branches Echinocactus seems to have sprung.

The genus Cereus seems to me an especially fruitful field for the study of internal anatomy on a comparative basis. It is rather evident, from a hasty glance at the great variety of forms, that many different lines of development begin in this vicinity, lines which, though at present somewhat unraveled from external character, could at least be well subjected to confirmatory tests upon structural grounds. This would be of especial value in searching for the origin of the various other genera, the supposed offspring of this group.

It must not be thought, however, that I wish to give too great importance in classification to the internal anatomy, preferring it to the external characters. Its principal use is confirmatory. Too many internal variations spring up independently in several forms to make them alone sufficiently reliable. There must be some gradual internal metamorphosis, however, to correspond with the external. In what deep-seated organs this is shown, and what parts are more easily influenced by environmental

[8] Engelmann's Cact. Mex. Boundary.

conditions, can best be determined by careful study. At present I am inclined to believe that the most permanent and satisfactory evolutionary characters will be found in the fibrovascular system. When its metamorphosis is fully understood a far clearer insight can be gained, not only into the interrelationships of these peculiar and at present much confused groups of plants, but also into the steps by which they became gradually accustomed to their present rigorous habitat.

HARVARD UNIVERSITY.

Later investigation shows that the statement (p. 42, line 17 ff.) regarding periodicity of growth is incorrect. The smaller amount of growth noted was due to the drouth of the summer of 1900. In spite of their storage facilities, these plants are apparently affected by temporary unfavorable conditions to a degree almost surprising.

BRIEFER ARTICLES.

NOTE ON BASILIMA AND SCHIZONOTUS OF RAFINESQUE.

See p 440-

As *Basilima* of Rafinesque is still considered by many botanists to be the valid generic name for the genus based on *Spiraea sorbifolia* Linn., it will probably not be uninteresting to point out that it has no claim to the priority attributed to it. Until 1891 the genus has been best known under the name Sorbaria, proposed first by Séringe (DC. Prodr. **2**: 545. 1825) as a section of Spiraea, and given generic rank by A. Braun (Ascherson's Flor. Brandenb. 177. 1864). In 1891, however, E. L. Greene (Flor. Francisc. 57) and O. Kuntze (Rev. Gen. Plant. **1**: 215) almost simultaneously took up the Rafinesquean name Basilima for Sorbaria, and claimed priority for it on the ground that Rafinesque in his *New Flora of North America* (**3**: 75. 1836) quotes "Raf. 1815" as authority for his Basilima, but neither of them seems to have seen or even known the original place of publication. If they had seen it, they would have been aware that Basilima of 1815 is a *nomen nudum* pure and simple, and therefore not entitled to priority. Rafinesque unfortunately makes no mention of the place of publication, but after some searching I was fortunate enough to find the name in his *Analyse de la nature ou tableau de l'universe et des corps organisés*, a very rare book of 244 duodecimo pages, published in Palermo in 1815. It aims to give a short review of the whole range of natural history, and contains on page 173, under the order Rhodanthia, the following passage reproduced here verbatim et literatim:

> 5. Famille Spireadia. Les Spiréades. Etamines hèterines, perigone double, l'interne corollé, fruits capsules dehiscentes interieurement. G. 1. Spirea L. 2. Rhodalix R. sp. do. 3. Drymopogon R. sp. do. 4. Basilima R. sp. do. 5. Filipendula T. 6. Gillenia Mench.

From this enumeration it is impossible to gain any idea which species of Spiraea Rafinesque intended to include under his genera Rhodalix, Drymopogon, and Basilima, and he could therefore not claim priority for it, as he does, and quote as synonyms of his Basilima two names published before 1836. The older of these two names is

the Sorbaria of Séringe mentioned above, the second the Schizonotus of Lindley, published in 1830 in his *Introd. Nat. Syst. Bot.*, p. 81, and (under no. 703) in Wallich's *Catalogue*, which appeared about the same time. In both places, however, the name is mentioned only as a synonym, and it seems doubtful whether this ought to be considered as a real publication of a generic name. The passages in both publications read as follows: Wallich, Cat., p. 21, "no. 703. Spiraea Lindleyana Wall. Schizonotus Lindl. (gen. nov. Spir. sorbifoliam amplectens);" Lindl. Introd. Nat. Syst. Bot. ed. 1, p. 51, "Rosaceae. . . . . The fruit of Spiraea sorbifolia (Schizonotus m.) is capsular." In the second edition of his work, however, which appeared in 1836, Lindley enumerates (p. 145) Schizonotus as a genus, and characterizes it (p. 441) by mentioning *Spiraea sorbifolia* as the type. In the same year, Rafinesque published in *New Flor. N. Am.* (**3**: 75–76) the genus Basilima, with descriptions of two species, *B. sorbifolia* and *B. pygmaea*. As it seems impossible to ascertain which of the two works was really published first, the genus Basilima ought to be preferred, since it had specific names attached to it, while Schizonotus had none and received none until 1840, when Lindley (Bot. Reg. **26**: misc. 71) proposed the name *Schizonotus tomentosus* for *Spiraea Lindleyana* Wall.

Those botanists who object to Sorbaria because it was published only as a sectional name must therefore choose between Schizonotus and Basilima. It would be very unfortunate, however, to revive the name Schizonotus, since it was applied afterwards and has been in use for two other genera, first to a genus with *Spiraea discolor* Pursh as the type by Rafinesque, who entirely misunderstood a note of Lindley in *Bot. Reg.* **16**. 1830 in the text to *pl. 1365*, and says in *New Flor. N. Am.* **3**: 75 under Schizonotus "I have adopted this genus and name on the suggestion of Lindley, who proposed to unite to it the next [Basilima], but the habit is too different." But Lindley did not say this. He only mentions there on the same page these two species of Spiraea, together with some others, and the name Schizonotus. The second application of the name Schizonotus was by A. Gray (Proc. Amer. Acad. **12**: 66. 1876) to a genus of Asclepiadaceae, which was subsequently changed by E. L. Greene to *Solanoa;* while on *Spiraea discolor* the name *Holodiscus* was bestowed first by C. Koch, who proposed it in his *Dendrologie* (**1**: 309. 1869) as a subgenus of Spiraea, which was afterwards given generic rank by Maximowicz in his excellent monograph of the Spiraeaceae (Acta Hort. Petr. **6**: 253. 1879),

without either of the authors being aware of the existence of a *Schizonotus* Raf.

In *Index Kewensis* the Schizonotus of Lindley and of Rafinesque are not discriminated, but wrongly united under *Schizonotus* Lindl.; while *Schizonotus* Gray is enumerated as valid. Also the *Thecanisia discolor* Raf. is there erroneously made a synonym of *Spiraea discolor*, but it is an herbaceous plant allied to *Ulmaria rubra*, and probably based on escaped plants of the European *U. pentapetala* Gilib. (*Spiraea Ulmaria* Linn.)

It will be clear that on account of priority as well as of usage the name Sorbaria ought to be considered as the valid name, with *Schizonotus* Lindl. (1830) and *Basilima* Raf. (1836) as synonyms; while *Holodiscus* (Koch) Maxim. should be substituted for *Schizonotus* Raf., at least by those who follow the Rochester rules in accordance with article IV, and by others on account of expediency, to avoid all possible confusion with the other two genera, of which one is liable to be revived under certain rules of nomenclature.

Sorbaria of course will not enter into the American flora if Chamaebatiaria is considered as constituting a distinct genus, which it may be, though structural differences in flower and fruit are hardly present. In foliage and habit, however, it is strikingly different. The observation of Rafinesque that *Sorbaria sorbifolia* is a native of Northwest America seems not to be based upon any reliable source, at least it has not been found again on American soil, neither has *S. grandiflora* Maxim. (*Basilima pygmaea* Raf.), which was suspected by him to occur probably somewhere in the same region.—ALFRED REHDER, *Arnold Arboretum.*

POTAMOGETON POLYGONIFOLIUS IN NEWFOUNDLAND.

THE only note of the occurrence of this species in North America I know of is contained in Linnaea 2:216. 1827; where Chamisso has the following: "Hujus loci forsitan est: Potamogeton de St. Pierre Miquelon près Terreneuve in Herb. Brogniart, sed major." I have not seen the specimens in Brogniart's herbarium, which is at Paris.

In the *Journal of Botany* for June of this year, I record it from Sable island, about 100 miles off the coast of Nova Scotia. I have now to record it from Newfoundland, from whence I find specimens the Kew herbarium from the herbarium of Harvard University.

As this was gathered by American botanists I record it in an American journal, the more so as I wish to point out that there is some hope it may occur in Massachusetts in the neighborhood of where *Calluna vulgaris* L. occurs. The label of the Newfoundland specimens runs thus:

No. 231. *P. heterophyllus* Schreb. Muddy banks of brooks. Whitbourne. 17. 8. 1890. Coll. B. L. Robinson (and) H. Schrenk. Distributed from the Herbarium of Harvard University.

The two specimens are not *P. heterophyllus*, however, but *P. polygonifolius* in its usual "heath" form. As this is the first time it appears in American books it may be well to give the synonymy and distribution.

P. POLYGONIFOLIUS Pourr. in Acad. Toul. 3:325. 1788.

P. oblongus Viv. Anal. Bot. 2:102. 1802; also in Fragm. Fl. It. 1:1. *pl. 2.* 1808.

P. plantago Batard, Fl. dep. Maine et Loire 64. 1809.

P. parnassifolius Schrad. (ined. 1818) ex Mert. et Koch, Deut. Fl. 1:839. 1823.

P. uliginosus et *affine* Boenning ex Cham. in Linnaea 2:216. 1827.

P. paludosus Bory in sched. ex Cham. *l. c.*

P. natans intermedius Mert. et Koch, Deut. Fl. *l. c.*

P. microcarpus Reut. et Boiss. Diag. Pl. Nov. Hisp. 2:24. 1842 is a variety, or perhaps a subspecies.

DIST. EUROPE: Iceland to Piedmont, and Holland to Russia.

ASIA: Siberia altai (Led.) to Mongolia, and China to India and Japan.

AFRICA: Morocco to Transvaal? (Wilms), and Madagascar.

AUSTRALIA: New Zealand.

The species varies greatly, the var. γ *ericetorum* Syme, Eng. Bot. ed. 3, 9:28. 1869, representing the small heath form, often growing in mud, the var. β *pseudo fluitans* Syme, *l. c.*, the other extreme, *i. e.*, a fluitant form often in deep or running water with thin translucent submerged leaves and coriaceous upper leaves.

It may be distinguished from *natans* by the small fruit, the blunt stipules, the base of the leaves never having the flexible joint of *natans* (there are no "strings" in the leaf of *polygonifolius*) which enables that species to turn its leaves in any direction; from *heterophyllus* by the style being central, by the nervation of the leaves, etc.

I hope to send specimens of this species to various American botanists next fall, so that they may know it and keep it in view.—ARTHUR BENNETT, *Croydon, England.*

A NEW SPHAERALCEA.

Sphaeralcea martii, n. sp. — Perennial, low and rather spreading, 15 to 30^{cm} high: leaves and stems whitish-green with an excessively dense white stellate pubescence: leaves subhastate, with broad short basal lobes, crinkled, edges coarsely irregularly serrate, about 27^{mm} long and 15^{mm} wide; petioles about 10^{mm} long: flowers large, 23^{mm} in diameter, in clusters of six or seven at the ends of the branches; pedicels 5 to 7^{mm} long: calyx 11^{mm} to tip of lobes, 4^{mm} to base of lobes: petals fully 15^{mm} long and broad, rather pale but vivid scarlet (dull pink in dry material): anthers orange; pollen bright orange: styles crimson: fruit maliform, densely white-hairy; carpels with very small beaks; ovules two in a cell.

Picacho mountain, Mesilla valley, New Mexico, in volcanic soil, March 25, 1900 (*Cockerell*); hills north of Picacho mountain, April 7, 1900, in fruit (*E. O. Wooton*). A specimen is in Herb. U. S. National Museum.

Differs from *S. subhastata* in its pointed leaves, clustered flowers, deeply cleft calyx with long acuminate lobes, and especially in its early flowering, wherein it resembles the annual species. The only flowering date I find reported for *S. subhastata* is in August.—T. D. A. COCKERELL, *East Las Vegas, N. M.*

CURRENT LITERATURE.

BOOK REVIEWS.

Handbook of systematic botany.

THE first volume has appeared of a handbook on systematic botany by Dr. Richard R. v. Wettstein,[1] of the University of Vienna. We do not often have texts that confine themselves so closely and accurately to the subject as does this very excellent outline of classification. Its aim is to give a general view of the plant kingdom with especial reference to the evolution of phylogenetic lines. Prefacing the special parts, which deal specifically with the groups of plants, there is a general treatment of a number of interesting topics. There is first an historical account of the development of systematic botany, which one wishes were longer. This is followed by discussions of the conditions which lead to the development of lines (phyla) of ascent and the sort of evidence of value in the determination of the same. There is an excellent account of the methods of systematic botany and the data of most value in studies on classification. Admirable illustrations are presented of analogous and homologous organs, rudimentary structures and juvenile forms. Finally the author considers the origin of new forms on the supposition of phylogenetic evolution, or in other words the principles of Darwinism. This is in the main a simple account of evolutionary factors, and is particularly good in the treatment of adaptation and the changes in species effected directly by environment. At this point the author shows clearly the importance of the so-called ecological factors in the definition and adjustment of species.

Following the short general part, which is only forty-four pages long, comes the special part that will deal entirely with the various groups.

The present volume ends with the thallophytes, but we are promised the completion of the subject next year. The author divides the plant kingdom into seven branches, as follows: 1) Myxophyta, 2) Schizophyta, 3) Zygophyta, 4) Euthallophyta, 5) Phaeophyta, 6) Rhodophyta, and 7) Cormophyta. The success of a division into great groups depends primarily upon the balance maintained, and this one has some peculiar disadvantages in its lack of symmetry. It will hardly be questioned that branches 1, 2, 3, 5, and 6 are well differentiated phyla or closely related groups of phyla. But this simplicity is all out of proportion to the complex conditions presented in the

[1] WETTSTEIN, RICHARD V.: Handbuch der systematischen Botanik. Vol. I. 8vo. pp. vi + 201. *figs.* 762. Leipzig: Franz Deuticke. 1901.

Euthallophyta and Cormophyta. Passing to the detailed subdivision of the main groups, the descriptions of orders and families and the illustrations are generally very good. We note that the Protococcaceae and Hydrodictyaceae are placed at the bottom of the large order Siphoneae, a position that is certainly open to question. The Charales are reduced to a suborder of the same group, which seems curious for so highly specialized and well defined a line of development. Among the fungi the Brefeldian system is followed in part, with much of the arrangement in Engler and Prantl; and the classification of the Phaeophyta and Rhodophyta is a brief outline of the latter work. These two groups are not given the attention they deserve.—B. M. DAVIS.

MINOR NOTICES.

HERMANN VON SCHRENK has published[1] his address on "Factors which cause the decay of wood," delivered before the Western Society of Engineers on February 6 last. It deals with such topics as structure, chemical nature, and decay of wood, fungi and structural timbers, and preventive measures.—J. M. C.

THE THIRD FASCICLE[2] of the list of the genera of seed plants, according to the system of Engler, has just appeared. The general character of the work was stated in this journal[3] in the notice of the first part. In the present signature 1352 genera are listed, bringing the number up to 3842, the list beginning with Lychnis (Caryophyllaceae) and ending with Geoffraea (Leguminosae).—J. M. C.

F. LAMSON-SCRIBNER[4] has published a revised edition of the second part of his *American grasses*, the first edition having been exhausted. The work has been entirely rewritten, the synonymy has been revised or extended, and the descriptions are much fuller. The two parts now contain illustrations and descriptions of 627 species, and are invaluable to those who would name grasses.—J. M. C.

CHARLES V. PIPER and R. KENT BEATTIE[5] have published a manual of the flora within a radius of about twenty-one miles around Pullman, Wash. This includes some twenty-four townships in Washington and eleven in

[1] Reprint from Jour. Western Soc. Engineers, May 1901.

[2] DALLA TORRE, C. G. DE, and HARMS, A.: Genera Siphonogamarum ad systema Englerianum conscripta. Fasciculus tertius (signatura 21–30). Small 4to. pp. 161–240. Leipzig: Wilhelm Engelmann. 1901. *M* 4.

[3] BOT. GAZ. 30: 67. 1900.

[4] American grasses. II. Revised edition. 8vo. pp. 349. Bulletin 17, Division of Agrostology, U. S. Department of Agriculture. 1901.

[5] The flora of the Palouse region. 8vo. pp. viii + 208. Published by the Washington Agricultural College and School of Science, Pullman. May 14, 1901.

Idaho, the region consisting mainly of rolling hills, destitute of trees and shrubs, and said to be quite typical of a large area in eastern Washington and western Idaho. These hills are generally called Palouse hills, and hence the title of the book. The manual certainly covers a region of very great interest in its unrivaled floristic riches. The keys and descriptions seem to be entirely adequate, and, checked as they have been by the large field experience of the authors, the manual must give as good a presentation of the flora as is possible at present. The nomenclature follows what are called the Kew and Berlin rules. Several new species are described, and the enumeration of species shows 14 pteridophytes, 9 gymnosperms, 114 monocotyledons, and 526 dicotyledons. The exceedingly varied conditions of the western mountain region will demand the publication of just such local manuals as this.—J. M. C.

THE LIVERPOOL Marine Biological Committee is doing good work in publishing short popular papers on the more interesting animals and plants in the general region of its activities, the Irish sea. The first three papers described animals, but the fourth, just issued, is on Codium,[6] a very interesting genus of the Siphonales. Following a general introduction, we have presented an account of the structure, reproduction, habits, and distribution of this alga. The life history is still incomplete in certain phases of reproduction. There are two forms of sporangia, one producing large green zoospores and the other small yellow elements, both however morphologically similar and biciliate. The larger green zoospores will germinate vegetatively, and the problems concern the fate and function of the small yellow bodies. They have been supposed to be sperms that should fuse with the green swarmers, but no unions have ever been observed. It is probable that the yellow zoospores are gametes, which under suitable condition will conjugate with one another. The authors of the paper suggest that the plant is becoming apogamous, a view that has support, further than the mere negative evidence, in the fact that the hypothetical gametangia at certain stages in their development may be reproductive. They are then adventitious buds, capable of growing out in a branching filament, which however appears to remain attached to the parent plant. The paper is illustrated with three very clear plates.—B. M. DAVIS.

NOTES FOR STUDENTS.

THE POLLEN tube in *Cucurbita Pepo* according to B. Longo[7] traverses the tissues of the funiculus and outer integument before entering the

[6] GIBSON and AULD: Codium. pp. viii + 18. *pls.* 3. L. M. B. C. Memoirs. IV. 1900.

[7] La mesogamia nella commune zucca (*Cucurbita Pepo* Linn.). Rendiconti della R. Accademia dei Lincei **10**: 168–172. 1901.

micropyle. The writer proposes the name *mesogamy* for this phenomenon.—CHARLES J. CHAMBERLAIN.

DR. N. WILLE[8] has begun a series of studies on the Chlorophyceae. The first paper contains numbers one to seven, and treats of the structure and phases in the life histories of *Sybidion Droebakense*, n. sp., Trochiscia, *Prasiola crispa*, *Ulothrix flacca* and several new species of Ulothrix, Pseudendoclonium, nov. gen., and certain species of Rhizoclonium. Four excellent plates accompany the text.—B. M. DAVIS.

AS A RESULT of experiments conducted in Maryland in the seasons of 1899 and 1900, C. O. Townsend finds[9] that celery blight, caused by the fungus *Cercospora apii*, can be prevented by the use of fungicides. The best results, apparently, are given by ammoniacal solution of copper carbonate, Bordeaux mixture being equally good as a fungicide but causing a slight stunting of the plants. To obtain the best results the first application should be made while the plants are still in the seed-bed, followed by other applications every week until cool weather, which checks the progress of the disease. —ERNST A. BESSEY.

THE SUBJECT of oat smut has received attention in two recent bulletins from the Illinois[10] and Wisconsin[11] experiment stations respectively. In both an estimate is given as to the annual loss in the state from this smut, about 14 per cent. for Illinois and about 6 per cent. for Wisconsin. The results of experiments with the hot water treatment at different temperatures are described in the former bulletin, the temperature found to be most efficient without injury to the germinative power of the grain being 135° F. for five minutes. Both bulletins discuss the formalin treatment, which is shown to be even more effective than that with hot water and somewhat more easy to perform. —ERNST A. BESSEY.

A SHORT NOTE on the abundant and destructive occurrence of the dwarf mistletoe, *Arceuthobium pusillum*, in the upper peninsula of Michigan is given in a recent bulletin from the Michigan Experiment Station.[12] In connection with the recent rediscovery of this interesting plant in New England, it is of

[8] WILLE, Dr. N.: Studien über Chlorophyceen. Med. f. d. biol. sta. v. Dröbak. no. 2. 1901.

[9] TOWNSEND, C. O.: Notes on celery blight. Bull. Maryland Agr. Exp. Sta. no. 74. pp. 167–182. *figs.* 1–7. May 1901.

[10] SHAMEL, A. D.: Treatment of oats for smut. Bull. Ill. Agr. Exp. Sta. no. 64, pp. 57–72. 6 *pls.* Urbana. March 1901.

[11] GOFF, E. S.: The prevention of oat smut. Special Bulletin, Wisconsin Agr. Exp. Sta. pp. 1–4. *fig.* 1. Madison. March 1901.

[12] WHEELER; C. F.: The geology and botany of the Upper Peninsula Experiment Station. Report of the Upper Peninsula Experiment Station for the year 1900. Bull. Michigan Agr. Exp. Sta. no. 186. pp. 17–28. 4 *pls.* Dec. 1900.

interest to note that in Michigan it is in some places so abundant as to kill out nearly every tree. The parasite itself is attacked by a fungus, *Wallrothiella arceuthobii* Peck, which apparently serves to keep it in check somewhat. In the same builetin are given notes on the trees and a list of the flowering plants growing on the station farm, as well as a list of the few of the commoner diseases of cultivated plants observed there.— ERNST A. BESSEY.

D. T. MACDOUGAL[13] has studied the bulbils which are formed in the axils of the aerial stems of *Lysimachia terrestris*, and regards them as representing a new category of propagative bodies. They are branches of restricted development, and are formed under conditions unfavorable for seed formation, diffuse light and low temperature apparently being the principal inciting causes. They are free from transpiratory organs of any kind, and resemble rhizomes in structure rather than aerial stems upon which they are borne. The "germination" of the bulbil occurs without any appreciable resting period, and is followed by the final stages of the differentiation of the stele, which was checked during the formation of the bulbil. The bulbil becomes the main axis of the new plant, and does not perish, except gradually, after the manner of a rhizome, into which it becomes converted.—J. M. C.

MUSHROOMS ARE DISCUSSED in two recent Experiment Station bulletins, one from Idaho[14] and the other from North Carolina.[15] Both give rules for avoiding poisonous fungi and analyses showing the food value of the edible species. This has been greatly overrated, for, compared with other foods, these fungi have not only a small heating power but have also a low nitrogen content. In the former bulletin a few species are popularly described with the aid of good half-tone plates, while in the bulletin from North Carolina all or nearly all of the edible species reported in the state are described, rather too technically it would seem. This technicality, combined with the lack of illustrations, leads one to fear that it will not be serviceable as a popular guide. The glossary, five pages in length, is no doubt necessary but might have been made more accurate. The definition of basidia as "cellular processes of certain mushroom-bearing spores" is probably a typographical error, but is plainly very misleading as it stands.— ERNST A. BESSEY.

THE THIRD EDITION of Sturgis's *Literature of Plant Diseases*[16] brings the

[13] Propagation of *Lysimachia terrestris*. Bull. N. Y. Bot. Garden **2**: 82–89. 1901.

[14] HENDERSON, L. F.: Mushrooms or toadstools: a natural food product. Bull. Idaho Agr. Exp. Sta. no. 27. pp. 27–54. *figs. 1–12.* Moscow. March 1901.

[15] HYAMS, C. W.: Edible mushrooms of North Carolina. Bull. N. C. Agr. Exp. Sta. no. 177. pp. 25–58. West Raleigh. Dec. 1900.

[16] STURGIS. W. C., Literature of plant diseases. A provisional bibliography of the more important works published by the U. S. Department of Agriculture and the agricultural experiment stations of the United States from 1887 to 1900 inclusive, on fungous and bacterial diseases of economic plants. Rep. Conn. Agr. Exp. Sta. for year ending Oct. 31, 1900. Part III. pp. 255–297. 1901.

bibliography of these diseases nearly up to date. The first edition appeared in 1893 and the second in 1897. It does not assume to be a complete bibliography, but a reference list "to enable the practical observer of plant diseases to ascertain what are the principal sources of information regarding the specific cause of a certain disease and the method of prevention as recorded in the publications of our own Department of Agriculture and of the various state experiment stations." As in the previous editions, the host plants are arranged alphabetically under their common names. A number of diseases formerly ascribed to parasitic organisms but since shown to be due to other causes are omitted, while many new host plants whose diseases have been studied in this country only in recent years are added, making the list now somewhat longer than before. The work is exceedingly useful not only to the practical worker but also to the specialist.—ERNST A. BESSEY.

THE ZYGOSPORE OF SPORODINIA was studied six years ago by Léger, who found that both gametes contain hundreds of small nuclei which become scattered in the mingling cytoplasm when the membrane separating the gametes breaks down. The nuclei near the periphery are much smaller than those nearer the center. At a later stage all the nuclei disappear, and at each pole of the zygospore there is found an "embryonic sphere" containing a large number of granules. The spheres increase in size and fuse with each other, and soon afterward numerous nuclei again appear. Gruber[17] has examined Sporodinia, and he also finds a large number of nuclei in the zygospore. The nuclei are more numerous at the periphery, but those at the periphery and those at the center are approximately alike in size. This condition persists for a long time, and subsequent stages were hard to follow. No fusion, division, or disorganization of nuclei could be established with any certainty. The presence of "embryonic spheres" is regarded as doubtful. On germination the nuclei appear in greater numbers and pass into the germ tube. Although the writer was not able to observe any fusion of nuclei, he believes that a fusion of nuclei at the center of the zygospore is very probable. —CHARLES J. CHAMBERLAIN.

THE EFFECT of fungicides upon the foliage of the peach is discussed by W. C. Sturgis in a recent report of the Connecticut Agricultural Experiment Station.[18] The experiments were made with various strengths of Bordeaux mixture, with a soda-Bordeaux, in which soda replaced the lime, with ammoniacal solution of copper carbonate, with copper acetate, and with potassium sulfid. The Bordeaux mixture was found to be injurious to the

[17] GRUBER, EDUARD: Ueber das Verhalten der Zellkerne in den Zygosporen von *Sporodinia grandis* Link. Ber. d. deutsch. bot. Gesell. **19**: 51–55. *pl.* 2. 1901.

[18] STURGIS, W. C.: Peach-foliage and fungicides. Report of the Connecticut Agricultural Experiment Station for the year ending October 31, 1900. Part III, pp. 219–254. *pls. 3–5.* 1901.

foliage except when very weak solutions were used. The soda-Bordeaux was also injurious, as was the ammoniacal solution of copper carbonate. Potassium sulfid, however, proved to be harmless, and at the same time to be a fairly good fungicide. Normal copper acetate solution was harmless but the subacetate caused injury. Careful comparative examinations of leaves of plants susceptible to injury by copper-containing fungicides, viz., peach, Japanese plum, and apricot, with leaves of plants not so injured, viz., European plum, apple, cherry, quince, and pear, failed to reveal any constant difference in the thickness of the leaves as a whole, or of the epidermis and the different layers of tissue, or in the size or number of the stomata. The susceptible leaves, however, had a very dense spongy parenchyma, with small intercellular spaces, while the non-susceptible leaves had this tissue very loose in texture.— ERNST A. BESSEY.

CLEISTOGAMOUS FLOWERS[19] are found in nearly all violets, but are especially typical in *Viola odorata*. The normal flower which appears early in the spring has a handsome corolla, but it seldom produces good seed. The inconspicuous cleistogamous flowers which come later, usually after the normal flowers have disappeared, produce an abundance of good seed. The stamens are larger in the normal flowers than in the cleistogamous, but the size of the pollen grains is about the same in both. The structure of the anther wall is quite different, the normal anther having the usual endothecium with lignified thickenings, while in the cleistogamous flower the endothecial layer retains its nucleus and cytoplasm.

After the pollen is mature there is a resting period of various duration. Pollen tubes are then put out which penetrate the wall of the anther at its upper part where there is a region of small cells rich in protoplasm, a tissue comparable to the conductive tissue of the style. *Oxalis acetosella*, *Linaria spuria*, and *Leersia oryzoides* were also studied.

In typical cleistogamous flowers the pollen germinates within the pollen sac, and the structure of the anther wall is modified to meet the new mode of pollination. In Linaria and Leersia, where the pollen was not observed to germinate within the pollen sac, the anther wall has the same structure as in the normal flower.— CHARLES J. CHAMBERLAIN.

BULLETINS from the experiment stations of interest to botanists, and not heretofore mentioned in these pages, are as follows: A. S. HITCHCOCK and G. L. CLOTHIER (Kans. no. 87, pp. 29) write upon the "Native agricultural grasses of Kansas," with many illustrations and charts of distribution. H. GARMAN writes on the agricultural grasses of Kentucky, with some fine reproductions from photographs, and A. M. PETER supplies some chemical analyses, the two articles forming one bulletin (Ky. no. 87, pp. 68, *pl. 14*).

[19] DU SABLON, LECLERC: Recherches sur les fleurs cléistogames. Revue Générale de Botanique **12**: 305–318. *11 figs.* 1900.

F. A. WAUGH (Vt. no. 67, pp. 30) discusses hybridity among cultivated plums, and gives a systematic account of hybrid forms. Interesting trees of Vermont are described and figured by ANNA M. CLARK (Vt. no. 73, pp. 52), and those of Wyoming by AVEN NELSON (Wy. no. 40, pp. 52). W. W. ASHE (N. C. no. 175, pp. 8) gives technical diagnoses of 21 new species *Crataegus* and 8 new species of *Panicum*. E. E. BOGUE publishes "An annotated catalogue of the ferns and flowering plants of Oklahoma" (Okla. no. 45, pp. 48), D. A. SAUNDERS does the same for South Dakota (S. D. no. 64, pp. 127), and HENRY L. BOLLEY and L. R. WALDRON do the same for North Dakota (N. D. no. 46, pp. 91), all excellent beginnings toward complete floras of the respective states. C. F. WHEELER writes about the dwarf mistletoe in Michigan, with good reproductions from photographs, and on other topics (Mich. no. 186). L. H. PAMMEL describes the horse nettle (*Solanum Carolinense*), bind weed (*Convolvulus arvensis*), and ground burnut (*Tribulus terrestris*) as troublesome weeds in Iowa (Ia. no. 42.) D. A. BRODIE (Wash. no. 45, pp. 12) gives facts establishing the poisonous nature of the Oregon water-hemlock (*Cicuta vagans*).—J. C. ARTHUR.

FERTILIZATION IN *Ginkgo biloba* has recently been studied by Ikeno,[20] who gives a detailed account of phenomena from the cutting off of the ventral canal cell to the first division of the nucleus of the oospore. The nucleus of the ventral canal cell rapidly disorganizes, but in one instance it had enlarged *pari passu* with the nucleus of the oosphere. In preparations stained with methyl blue and acid fuchsin, the metaplasmic ground substance of the nucleus stains red, and the chromatin, which forms a small, irregular, granular mass, also takes the red, while the nucleoli stain blue. The nucleus then undergoes a great change in structure, so that the metaplasm and chromatin can no longer be distinguished from each other. The further development of the nucleus of the oosphere agrees with the description of the corresponding phenomena in *Pinus Laricio* as described by the reviewer in 1899. The tube nucleus and the nucleus of the stalk cell disorganize within the pollen tube and do not enter the oosphere, and it is very probable that only one of the male cells is discharged, the other disorganizing without being able to enter. The nucleus of the male cell slips out from the cytoplasm mantle before fusing with the nucleus of the oosphere. The mode of fusion is like that already described for *Cycas revoluta*, that is, the male nucleus gradually penetrates the egg nucleus before losing its own membrane. At the time of fusion the sex nuclei are very unequal in size, the female being about ten times as large as the male. The behavior of the chromatin during fusion is not described. The spindle in the first division of the fusion nucleus is very broad and multipolar, and is never parallel with the longitudinal axis of the

[20] Contribution à l'étude de la fécondation chez le *Ginkgo biloba*. Ann. Sci. Nat. Bot. VIII. 13: 305–318. *pls. 2–3*. 1901.

oospore. In the case figured the spindle is transverse. Fertilization occurs while the ovules are still on the tree.—CHARLES J. CHAMBERLAIN.

DOUBLE FERTILIZATION in *Zea Mays*, which has been suspected for some time, and which is believed to be the cause of xenia, is described in a recent paper by Guignard.[21] The mature pollen grain contains, besides the vegetative nucleus, two very small elongated male cells, each in the form of a slender rod, curved or straight, and the ends often pointed. The cytoplasm of these cells is much reduced and difficult to distinguish, and their nuclei appear almost homogeneous. The synergids and oosphere are large, the former showing near the tip a conspicuous longitudinal striation, especially in material fixed in absolute alcohol. The nucleus of the oosphere is very large and contains much chromatin, and the cytoplasm is usually highly granular and much massed together at the time of fertilization. Near the oosphere, sometimes in the median plane, sometimes near the side of the embryo sac, are the two polar nuclei which do not fuse before fertilization, and have relatively large nuclei and a small amount of chromatin. As many as a dozen multinucleate cells may be found in the much narrowed antipodal end of the embryo sac. The pollen tube, after penetrating the embryo sac, usually seems to discharge its contents into one of the synergids. In one instance the two elongated male cells were observed resting against the base of a synergid; under high magnification their chromatin was distinct. One of the male cells unites with the oosphere, the other with the polar nuclei, which it binds together. Fertilization proceeds with such great rapidity that it could be observed in very few preparations. In general, the ovules at the base of the ear are first fertilized, and in hybrids many ovules are not fertilized. After fertilization one of the synergids usually persists for a time, with the contents finely granular and refractive. Division of the definitive nucleus proceeds so rapidly that the author was not able to follow the course of cell division. The first two nuclei of the endosperm are large, each one having an enormous nucleolus and many smaller nucleoli. It is to be regretted that no figures are given.—W. J. G. LAND.

THE EMBRYOLOGY of the Balanophoraceae presents many puzzling peculiarities. Accounts are somewhat divergent, but whether the divergence is due entirely to variation in the processes still remains to be seen. Writers agree that there is no ovule or placenta in Balanophora but that the megaspore is situated in a tissue at the base of a prolongation incorrectly termed a "style." Van Tieghem (1896) found that in *B. indica* the polar nuclei do not fuse and that fertilization occurs at the antipodal end of the sac as often as at the upper end.

According to Treub (1898) in *B. elongata* the megaspore germinates in the usual manner. The polar nuclei, however, do not fuse but each divides

[21] La double fécondation dans le mais. Jour. Bot. **15**: 1–14. 1901.

independently. The egg apparatus breaks down and there is no fertilization, but an embryo develops from one of the cells of the endosperm. Lotsy (1899) investigated *B. globosa* and supported Treub in every particular, including the peculiar origin of the embryo.

Chodat and Bernard have recently investigated *Helosis guayanensis*.[22] The archesporial cell becomes the megaspore directly without cutting off a tapetal cell or giving rise to a row of potential megaspores.

The jacket or "tapetum" surrounding the embryo sac is sporogenous tissue. The two daughter nuclei resulting from the first division of the nucleus of the megaspore are quite different in appearance, the one at the upper end of the sac staining much more deeply. This nucleus gives rise to the egg, two synergids, and a polar nucleus in the usual manner. The other nucleus stains faintly and rarely divides at all, but soon degenerates so that no antipodals or polar nucleus are formed. According to Van Tieghem, the egg is fertilized in Helosis and Balanophora.

The present writers find that in Helosis the egg becomes large, but also becomes very weak and feeble in appearance, so that while they were not able to prove or disprove the occurrence of fertilization, they believe that the feeble condition of the egg together with the position of the embryo in the endosperm favor Treub's view that the embryo arises apogamously from the endosperm.—CHARLES J. CHAMBERLAIN.

ITEMS OF TAXONOMIC interest are as follows: E. P. BICKNELL (Torreya **1**: 25–28. 1901) has described a new Triosteum (*T. aurantiacum*) from the northeastern United States.—*Pl. Bakerianae* **2**: 1–42. 1901 contains Baker's collection of 1899, from fungi to grasses. Numerous new fungi are described by F. S. EARLE.—ALICE EASTWOOD (Bull. Torr. Bot. Club **28**: 137–160. *pls. 15–20.* 1901) has published upon some small-flowered species of Nemophila from the Pacific coast, describing twenty-six new species.—M. A. HOWE (*idem* 161–165) has described a new Riccia from Georgia.—E. P. BICKNELL (*idem* 166–172) has revised the eastern species of Teucrium, recognizing six species and describing four as new.—P. A. RYDBERG (*idem* 173–183), in further studies of the Potentilleae, describes new species of Potentilla (8), Horkelia, and Drymocallis.—W. A. SETCHELL (Zoe **5**: 121–129. 1901), in his "Notes on Algae," has described two new genera of Laminaceae (*Hedophyllum* and *Pleurophycus*) and a new genus of Dumontiaceae (*Weeksia*), besides several new species in other genera.—A. ENGLER (Bot. Jahrb. **36**: 29–126. 1901), in his 21st contribution to the African flora, presents the following papers: Fungi by P. HENNINGS, who describes numerous new species and two new genera, *Fistulinella* (Polyporaceae) and *Lactariopsis* (Agaricaceae); Algae by W. SCHMIDLE; a revision of Schrebera (Oleaceae) by E. GILG;

[22] CHODAT, R., and BERNARD, C.: Sur le sac embryonnaire de l'Helosis guayanensis. Jour. de Botanique **14**: 72–79. *pls. 1–2.* 1900.

Leguminosae by H. HARMS, *Scorodophloeus*, *Rhynchotropis*, and *Schefflerodendron* being new genera; Myrsinaceae by E. GILG; Amarantaceae by G. LOPRIORE, *Argyrostachys* being a new genus; Acanthaceae by G. LINDAU; Caricaceae by I. URBAN, *Cylicomorpha* being a new genus; and Gramineae by R. PILGER.—CARL MEZ (*idem* Beibl. **30**: 1–20. 1901) has described numerous new species of Bromeliaceae and Lauraceae.—I. URBAN (*idem* 27–38) continues his papers on new American plants.—A. ENGLER (*idem* 42) has described a new genus of Araceae (*Protarum*) from the Seychelles.—B. D. GILBERT (Fern Bulletin **9**: 27. 1901) has described a new variety of *Botrychium ternatum* (*Oneidense*) from central New York.—ACHILLE FORTI (Ber. deut. bot. Gesell. **19**: 6–7. 1901) has published a new genus (*Heteroceras*) of marine Peridineae.—W. SCHMIDLE (*idem* 20–24) has described a new genus (*Coccomyxa*) of the Protococcoideae.—C. S. SARGENT (Rhodora **3**: 71–79. 1901) has described six new species of Crataegus from the Province of Quebec near Montreal.—G. P. CLINTON (*idem* 79–82) has described two new smuts on *Eriocaulon septangulare*.—E. L. GREENE (*idem* 83–84) has segregated from *Eupatorium ageratoides* the bulk of the New England and northern forms that pass under that name, and called the species *E. boreale*.—C. DE CANDOLLE (Bull. Herb. Boiss. II. **1**: 353–366. 1901) has published an account of the Brazilian Piperaceae and Meliaceae collected by W. Schwacke, including descriptions of numerous new species.—R. CHODAT (*idem* 395–442), in continuing his account of the Hassler collection from Paraguay, has described numerous new species in various families.—W. TRELEASE (Rep. Mo. Bot. Gard. **12**: 77. *pl. 34*. 1901) has described a new cristate variety of *Pellaea atropurpurea* from Missouri.—J. W. TOUMEY (*idem* 75–76. *pls. 32–33*) has described a new Agave from Arizona. H. M. RICHARDS (Bull. Torr. Bot. Club **28**: 257–265. *pls. 21–22*. 1901) has described a new genus *Ceramothamnion*) of red algae from Bermuda.—P. A. RYDBERG (*idem* 266–284) has published a fifth fascicle of new species from the Rocky mountain region, among them being *Piperia*, a new genus of orchids.—J. K. SMALL (*idem* 290–294) has published a third paper on the shrubs and trees of the southern states, including a revision of the southeastern species of *Ptelea*.—In Torreya (**1**: 54–55. 1901) J. K. SMALL has published a new *Cornus* from Kentucky, and N. L. BRITTON a new *Crataegus* from Washington.—F. LAMSON-SCRIBNER and E. D. MERRILL (Rhodora **3**: 93–128. 1901) have published a revision of the New England species of *Panicum*, recognizing thirty-five species.—J. K. SMALL (Bull. N. Y. Bot. Garden **2**: 89–101. 1901) has published a synopsis of the Mimosaceae of the southeastern United States. He recognizes fourteen genera, and among them *Siderocarpos* and *Havardia* have been separated from Pithecolobium as new.—M. A. HOWE (*idem* 101–105. *pl. 14*) has published an enumeration of the liverworts collected in the Yukon Territory by R. S. Williams in 1898–9, including a new species of

Scapania. MR. WILLIAMS himself (*idem* 105–148. *pls. 15–24*) has enumerated the mosses, including a new genus (*Bryobrittonia*) closely related to Tortula and Desmatodon, and a number of new species. L. M. UNDERWOOD (*idem* 148–149) enumerates the pteridophytes; while the seed plants are presented by N. L. BRITTON and P. A. RYDBERG (*idem* 149–187), numerous new species being described.—P. A. RYDBERG (*idem* 187–233 *pls. 25–33*) has given an account of the oaks of the continental divide north of Mexico, recognizing twenty-nine species, nine of which are new.—J. M. C.

PROFESSOR A. B. MACALLUM[23] has recently added an interesting contribution to the cytology of certain so-called non-nucleated organisms. His work is divided into three parts, each dealing with a separate group of low organisms—the Cyanophyceae, Beggiatoa, and the yeast cell—and was undertaken with the hope of throwing some light on the origin of the cell nucleus, and to obtain data to determine the morphological character of the primal life organism. In his investigations Macallum not only used the ordinary cytological methods, but microchemical tests were also employed to advantage. Picric acid and corrosive sublimate afforded the best results as fixing fluids. The stains that gave the best differentiation were Ehrlich's and Delafield's haematoxylin, Czokor's alum cochineal, saffranin, eosin, picro-carmin, and methylen-blue. The microchemical methods employed for obtaining the reactions for "masked iron" were practically the same as those used in his earlier work published in 1896. The iron liberated by sulfuric acid alcohol was converted into Prussian blue, the trichomes were then stained with a picro-carmin solution for twenty-four hours, when the cyanophycin granules acquired a deep red color which contrasts markedly with the Prussian blue tint of the iron-holding granules. The results obtained on the Cyanophyceae are briefly as follows. The cell consists of two portions, the central body and the peripheral zone holding the pigment. There is no evidence of the presence of a special chromatophore. There are two types of granules present in the cell. The one stains with haematoxylin, contains "masked iron" and organic phosphorus, and therefore resembles chromatin. The other type is found in the peripheral layer, and chiefly adjacent to the cell membrane. It stains with picro-carmin, and is free from organic phosphorus and "masked iron." It is probably a proteid. There is no nucleus or any structure which resembles a nucleus in the Cyanophyceae. In Beggiatoa there is no differentiation of the cytoplasm into a central body and a peripheral layer, such as Bütschli describes. The compounds of "masked iron" and organic phosphorus are uniformly diffused throughout the cytoplasm in the threads. In the "spirilla," "comma," and "coccus" forms the cytoplasm shows characters like those of the threads, but there are also granules present

[23] On the cytology of non-nucleated organisms. University of Toronto studies. Physiological series 2. 1900.

which give a slight reaction for "masked iron" and organic phosphorus, and therefore are considered analogous to chromatin. No specialized chromatin-holding structure in the shape of a nucleus was found in any of the forms of Beggiatoa studied. In his studies on the yeast cell, Macallum finds that the cytoplasm takes a stain with haematoxylin and gives a diffuse reaction for "masked iron" and organic phosphorus. In addition to the chromatin-like substance diffused throughout the cell, there is usually present a homogeneous corpuscle. This is not considered to be a nucleus, although held to be such by other investigators. The chromatin-like substance in Saccharomyces is soluble in artificial gastric juices, thus differing from the chromatin of the higher plant and animal cells. The paper is illustrated by a colored lithographic plate. It is a valuable and highly interesting addition to the literature of this important problem.—A. A. LAWSON.

NEWS.

DR. G. T. MOORE, of Dartmouth College, has been appointed phycologist in the Department of Agriculture.

DR. J. C. ARTHUR sailed July 6 and will spend July and August in Europe with his bride. He will attend the botanical conference at Geneva in August.

DR. J. B. OVERTON, who received his doctorate in June from the University of Chicago, has been appointed professor of botany in Illinois College, at Jacksonville.

Mr. A. A. LAWSON, fellow in botany at the University of Chicago during the past year, has been appointed assistant in botany in Leland Stanford Junior University.

M. HENRI PHILIBERT, honorary professor in the Faculty of Letters, distinguished for his bryological studies, died at Aix on the fourteenth of May, in his seventy-ninth year.

PROFESSOR G. J. PEIRCE, of Leland Stanford Junior University, takes charge of the botanical work during the summer at the Hopkins Seaside Laboratory near Pacific Grove, California.

WE LEARN from *Science* that a memorial tablet of the late Thomas Conrad Porter, long professor of botany in Lafayette College, was unveiled in connection with the recent commencement exercises.

PROFESSOR C. E. BESSEY, after attending the meeting of the American Association for the Advancement of Science in Denver, will go to the Pike's peak region to join the University of Nebraska camp for a month, to study the mountain flora.

THE REGENTS of West Virginia University have abolished the professorship of botany in that institution without notice to the present incumbent, Dr. E. B. Copeland. Dr. Copeland will spend the summer at the Cold Spring Biological Laboratory.

MR. H. N. WHITFORD, assistant in botany in the University of Chicago, has been appointed a collaborator of the Bureau of Forestry for the study of the ecology of the forest. He is to carry on work this summer in the neighborhood of Flat Head lake, Montana.

DR. H. C. COWLES, of the University of Chicago, will conduct a field party during August in northwestern Montana. The party will number about

twenty, and the work will be along ecological lines. Most of the work will be in the neighborhood of Flathead lake, where the state biological station is located.

THE AMERICAN ASSOCIATION for the Advancement of Science will meet at Denver, Colorado, August 23–28, 1901. A preliminary program of the meetings of section G (Botany) will be issued about July 15. One day will be given up to a joint session with the Botanical Society of America. It is also planned to devote one day's program to the subject "Adaptations of desert plants."

DR. F. E. CLEMENTS is to spend the summer in the Pike's peak region in Colorado, engaged in ecological studies of the flora. He will be accompanied by a party of botanists from the University of Nebraska, numbering a dozen or more. Instruments for accurate observation of ecological factors have been provided for the party.

THE LATEST *Bulletin* of the New York Botanical Garden, issued May 27 last, contains much information of interest to botanists. The planting of the grounds is proceeding rapidly, and the present showing of species grown during the year is as follows: herbaceous grounds 2300, fruticetum 450, salicetum 40, arboretum 220, and viticetum 60. During the year 48,895 specimens have been added to the herbaria.

JOHN J. THORNBER has been appointed special botanical collector for the University of Nebraska for the summer of 1901. He is to accompany the field party of the United States Forestry Division now at work in Nebraska, and is to act as its botanist, at the same time being the botanical representative of the university. In addition to the collection of specimens, he is to make careful ecological studies throughout the territory traversed.

UNDER A commission from the United States government, Dr. H. von Schrenk, of the Shaw School of Botany, is to spend the summer in Europe, in an investigation of the problems connected with the decay of railroad ties on the principal roads, this work being done in connection with an extensive series of investigations into the same subject which he is undertaking for the Department of Agriculture and in which the principal American railroads are cooperating.

ON MAY THIRTIETH there occurred the unveiling of a memorial tablet to Asa Gray in the Hall of Fame of the University of New York. The ceremony was committed to the Botanical Society of America. Most appropriately Dr. B. L. Robinson, professor of systematic botany in Harvard University and curator of the Gray herbarium, and Dr. N. L. Britton, director of the New York Botanical Garden, were appointed to act as representatives of the society, Dr. Robinson unveiling the tablet.

SUMMER FIELD WORK will be undertaken by the staff of the School of Botany of the University of Texas as follows: One party will make an exploration

of Padre island between Corpus Christi and Brownsville. A member of the staff will accompany a party conducting the mineralogical survey, which will make an expedition into the trans-Pecos region of the state. A party of students will collect plants and study ecological questions from Cuero westward toward the Pecos river. These various expeditions should do much in making known the state botanically.

THE BOTANICAL staff in the State University of Iowa has organized a summer school of botany to be conducted along the shores of Lake Okoboji in northern Iowa. The region is not only one of the most delightful of western summer resorts, but has unusual attractiveness for the naturalist. The university offers this year to students of botany a laboratory with all essential apparatus and libraries. Abundant boats put all the lakes into easy communication with one aaother, and in addition the laboratory will have its own equipment for the use of students. Professor Shimek will act as director and will be aided by special assistants. It is expected that hereafter the work in botany for the summer session of the State University of Iowa will all be conducted at Okoboji.

DR. J. N. ROSE left about the 20th of June for his third botanical trip to Mexico. He expects to go first to the City of Mexico, working out from this point as a base southward towards Acapulco and eastward toward Vera Cruz, probably ascending Mount Orizaba and Popocatepetl. The objects of his trips are to make a general botanical collection; to collect at type localities certain species of Humboldt, Galeotti, Schiede, and other early collectors; and to acquire information regarding the economic uses of Mexican plants, especially such as will supplement a second paper on the useful plants of Mexico, which is now nearly completed.

ERRATA in the last volume reported too late for inclusion in the usual list are as follows:

P. 109, line 4, after pellucido-punctata insert comma.

P. 121, line 8 from below, after corticata insert comma.

P. 392, line 14, for ERIGERON read ERIOGONUM.

P. 393, line 6, for TROGANUS read TIOGANUS.

P. 439, line 24, for position and negation read positive and negative.